# Studies on the wood decay by a soft rot fungus, Chaetomium globosum Kunze( Dissertation_全文 )

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CONTENTS

# INTRODUCTION

Wood is the only reproducible natural resource, and it must be preserved against unwanted deterioration. Especially, the decay of wood by fungi has become more and more important problem during the last years. The more the economic importance of wood losses due to decay by fungi increases, the more consideration must be given to methods of preserving wood through a better understanding of the organisms.

The correlation between fungi and decay in wood had been noticed from antiquity, but it was not until the second half of the last century that a group of the Basidiomycotina and a small group of the Ascomycotina were generally recognized as the causal agents of decay in wood[56]. These organisms were divided by Cartwright et al.[24] in 1931 into two groups, the brown rot fungi and the white rot fungi, according to the way in which the cell wall materials of wood were broken down. The idea was prevalent for the first half of the twentieth century that decay of wood was normally due to one or the other of these two groups of organisms. A particular type of fungal decay commonly called "soft rot" afterwards was not generally recognized in spite of a series of observations made between 1850-1950. Findlay and Savory[44] firstly demonstrated that the deterioration of timbers in cooling towers, which had been explained as only chemical phenomena, was in fact caused by a number of microfungi, i.e. members of the Ascomycotina and some Deuteromycotina. The term "soft rot" was coined by Savory[103] on account of the softening produced in the surface layers of wood by the action of these fungi. Following

their work it became currently accepted that this type of decay occurred in vastly varying situations, particularly in wood with high contents of water such as that in cooling towers, greenhouses, quays, poles in moist ground, etc. Stored pulpwood chips have also proven to be seriously attacked by this type of organisms[21].

In 1931, mode of action of soft rot fungi was first studied in detail by Bailey and Vestal[7]. They confirmed the existence of cylindrical cavities in the secondary cell walls, and noted that the conical ends of the cavity had a remarkably common appearance in a variety of timbers. Their work and the similar observation[9] made during this period were hardly realized to be important in the problem of wood decay as a whole. Since the establishment of soft rot type of attack in 1950[44] a number of papers[31,32,37,38,40,83,104] published in the 1950's and 1960's described the histology of soft rot attack, and presented the lists of fungi having the ability to introduce soft rot in hardwoods or softwoods in pure culture.

Microscopically, soft rot is generally quite different from either a white rot or a brown rot. Soft rot has been characterized by the presence of distinct elongated cavities within the middle layer of the secondary wall of fibres, tracheids and vessels[83]. Comparisons of the properties of soft rot with the brown- and white rots have also been made on the chemical- and mechanical properties of wood undergoing fungal attack. Chemical analysis of soft-rotted beech wood by Savory and Pinion [105] showed carbohydrate depletion with little lignin attack. That is the characteristic chemical situation of brown-rotted wood. The gradual

increase in alkali solubility of soft-rotted wood is more analogous to white rot than to brown rot[105]. The effect of a soft rot fungus, *Chaetomium globosum*, on the impact resistance of beech wood was compared by Armstrong and Savory[4] with that of white rot- and brown rot fungi. These results suggest that soft rot of beech by *C. globosum* is more closely comparable to a white rot than to a brown rot. However, changes in other strength properties of wood caused by soft rot fungi have not been studied yet in detail.

So far as the past evidences indicate, softwoods do not seem to be as readily attacked by soft rot fungi as are hardwoods. Although Savory[103] and Duncan[37] suggested that the higher content of lignin in softwoods was the main agent for the higher resistance of softwoods to soft rot fungi, none has yet proved entirely enough. An apparent wood preference has also been found for many of the brown rot- and white rot fungi. Brown rot fungi are associated most frequently with decay of softwoods and white rot fungi with decay of hardwoods. This dominant tendency has also not been fully explained yet.

Another series of investigations have proven that soft rot fungi are often more tolerant to fungicides than are wood-decaying Basidiomycotina[37], and that it is difficult to obtain the required fixation of the preservatives in the secondary cell wall[129], where the soft rot fungi are able to grow. However, ecological studies on soft rot fungi[83] revealed that they are of economic importance only when lack and excess of moisture or low dosage of treating preservatives inhibits the growth of the more vigorous Basidiomycotina. Soft rot fungi may also act as a

precursor of the wood-decaying Basidiomycotina in the deterioration of wood in ground contact[83].

Much remains to be done to achieve a better understanding of the nature of soft rot fungi. Particularly, the possible preference of soft rot fungi for hardwoods has aroused a considerable interest. Work along these lines could well throw light on the chemistry and biochemistry of wood decay, and on new ways of rapid disposal of wood waste by fungi.

Numerous species of fungi have been recorded as having the ability to induce soft rot in wood in pure culture. However, there have been conflicting reports as to the ability of fungal species to produce soft rot other than *Chaetomium globosum*. *C. globosum* was known to be a member of cellulolytic fungi which were important agents in the decomposition of paper, cotton and herbaceous plant materials. Since the fungus was demonstrated by Savory[103] as an important soft rot fungus occurring in cooling tower, many strains of the fungus have been isolated from vastly varying situations by several workers[37,38,44,103]. Furthermore, inferring from the past data, *C. globosum* seems to have the highest ability to degrade wood among soft rot fungi recorded. Therefore, *C. globosum* was used as a sole species of soft rotter in the present investigation.

The objects of the present study are three:
1) to obtain informations on the effect of *C. globosum* on the physical- and chemical properties of wood, for accomplishing a better understanding of the mechanisms of wood degradation by soft rot fungi, 2) to examine whether softwoods and hardwoods differ in their susceptibility to

*C. globosum*, and if the difference is found, 3) to demonstrate which factors are more responsible for the difference in susceptibility.

In Chapter 1, the composition of the medium and the size of the wood block employed throughout the accelerated decay tests in the present investigation are firstly discussed. In Chapter 2, changes in strength properties and infrared spectra of soft-rotted wood are compared with those of white- and brown-rotted woods. Utilization of carbohydrates by *C. globosum* is also described in the chapter. Results of the decay resistance tests for various wood species against *C. globosum* are mentioned in Chapter 3. Descriptions on the effects of some biological and chemical pre-treatments of wood on the wood-decaying capacity of the fungus are given in Chapter 4. Finally, in Chapter 5, removal of lignin from non- and partially delignified woods by *C. globosum* is investigated, and the role of lignin in different decay resistance of wood against the fungus is discussed together with the results obtained in the preceding chapters.

# CHAPTER 1 ANALYSES OF TESTING CONDITIONS PROVIDING A HIGHER WOOD-DECAYING CAPACITY OF CHAETOMIUM GLOBOSUM

The wood-decaying capacity of fungi, which is expressed mostly as the weight loss of wood specimen and occasionally as the retaining strength of wood in accelerated laboratory tests, often varies greatly with testing conditions. Although a great deal of experiment has been attempted to make the decay tests more reproducible and more reliable, no completely satisfactory test has been devised. However, as a means of understanding how wood preservatives function and how woods resist against fungal attack, several techniques have been standardized by an official agency of country. In Europe, the agar-block test has been used and specified in British Standard 838 and German Standard Din 52176. In North America and Australia, the soil-block test as outlined in AWPA Standard M 10-63 or ASTM Standard D 1413-61 is most widely used. In Japanese Standard, established as JIS Z 2119 in 1963, the sand-block test is adopted. Peptone-Malt Extract medium has been designated in this standard, but arguments are not yet decided on the concentration of carbon- and nitrogen sources in the medium.

Many investigators[25,27,34,41,43,45,50,62,88,139,140] have indicated several factors that are responsible for the great variation of the data wood species, fungal species, uniformity of test blocks, size of test blocks, setting of test blocks, temperature, test period, composition of medium, etc.

Test methods for soft rot fungi have not developed more than those

for Basidiomycotina fungi. For some of the tests a nutrient agar or soil is used as a substrate for growing of the fungus[39]. In Europe, blocks are sometimes buried in soil or in soil mixed with vermiculite[63].

The aim of this study is two: 1) to examine whether the Japanese Standard described above is applicable to test for a soft rot fungus, *Chaetomium globosum* Kunze, and 2) to find an appropriate composition of culture medium and an adequete size of test blocks which give a higher wood-decaying capacity of the fungus.

Effect of several factors which affect on the rate of wood decay is usually estimated by a classical combination method. In this method, the effect of a given factor could be estimated only when the other factors are equally fixed to a certain level. Although the results obtained by the method seem to be plausible, it is uncertain whether they never fluctuate under the different experimental conditions. Hence, reproducibility and reliability of the results are not always guaranteed by other laboratories. Long-term and expensive experiments are required to make the preciser estimate.

Design of experiment has been often introduced to investigate the reliable estimation of factorial effect more efficiently[50,51]. Characteristics of the method are[112]: randomization of order of experiment, orthogonal lay out of experiment, quantitative evaluation of factorial effect, and improved data-analysis.

In this chapter, analyses of the results of the two experiments by design of experiment are described.

## 1-1 Experiment I using Peptone-Malt Extract Medium[114)]

### Materials and Methods

Experiment I was carried out on four experimental factors with three levels using two wood species. These factors are : size of test block, fungal species, amount of peptone and amount of malt extract.

Test blocks were prepared from the sapwoods of *Fagus crenata* Blume and *Cryptomeria japonica* D. Don. The sizes of test blocks are: 1.5 (*tangential*) × 1.5 (*radial*) × 3.0 (*longitudinal*), 1.0 (*t*) × 1.0 (*r*) × 2.0 (*l*), and 0.5 (*t*) × 0.5 (*r*) × 1.0 (*l*) (cm). Test blocks will be called hereafter as "large" block, "middle" block and "small" block, respectively, according to the order of above size. *Chaetomium globosum* Kunze (IAM* 8059) was used as a test fungus. For comparison, a white rot fungus, *Coriolus versicolor* Quél. (FES** 1030), and a brown rot fungus, *Tyromyces palustris* Murr. (FES 0507), were used. Both fungi are designated as test fungi by JIS Z 2110-1963. Concentrations of peptone (Kyokuto Seiyaku, Japan) are:0.1, 0.3 and 0.5 (%). Concentrations of malt extract (Difco Laboratories, USA) are: none, 0.75 and 1.5 (%). Elemental analyses of these materials are shown in Table 1.

The nine experiments were laid out according to the orthogonal table $L_9(3^4)$ as shown in Table 2.

The decay test was carried out by the sand-block method. Cylin-

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drical glass bottles (9 cm in diameter and 16 cm in height), containing 350 g of quartz sand (ca. 30 mesh, Nakarai Chemicals, Japan) and 120 ml of nutrient solution, were screwed with metal caps. The bottles were autoclaved and inoculated with test fungi which had been previously allowed to cover the surface of the medium before the test blocks were inserted. Blocks were put in contact with the mycelial mat at radial surface. Two bottles which contained three blocks in each were used in

Table 1. Elemental analyses of peptone and malt extract used in Peptone-Malt Extract Medium.

| | C (%) | H (%) | N (%) |
|---|---|---|---|
| Peptone | 39.96 | 6.32 | 11.56 |
| Malt extract | 40.25 | 6.68 | 0.78 |

Table 2. Lay out of Experiment I using Peptone-Malt Extract Medium.

| Factor / No. | A | B | C | D |
|---|---|---|---|---|
| | Peptone (%) | Malt extract (%) | Fungus* | Test block** |
| 1 | 0.1 | none | P | small |
| 2 | 0.1 | 0.75 | V | middle |
| 3 | 0.1 | 1.5 | G | large |
| 4 | 0.3 | none | V | large |
| 5 | 0.3 | 0.75 | G | small |
| 6 | 0.3 | 1.5 | P | middle |
| 7 | 0.5 | none | G | middle |
| 8 | 0.5 | 0.75 | P | large |
| 9 | 0.5 | 1.5 | V | small |

* P: *Tyromyces palustris*, V: *Coriolus versicolor*, G: *Chaetomium globosum*.

** small: 0.5 × 0.5 × 1.0, middle: 1.0 × 1.0 × 2.0, large: 1.5 × 1.5 × 3.0 (cm).

each series of experiment. The composition of the nutrient solution is as follows:

$KH_2PO_4$ 3.0 g, $MgSO_4 \cdot 7H_2O$ 2.0 g, glucose 25.0 g, peptone, malt extract and distilled water 1000 ml.

The weighed test blocks were sterilized by fumigation with propylene oxide, and then exposed to fungi. The temperature was maintained at 28°C throughout five different incubation periods of 12-, 24-, 36-, 48- and 60-days. The decayed blocks were cleaned of mycelium and dried to constant weight in an oven at 65°C. After calculation of the loss of weight by decay, the decayed blocks were put to the test of compressive strength parallel to the grain. The test was made by a Shimadzu REH-10 multi-purpose testing machine following the JIS Z 2111.

## Results and Discussion

Analyses of variances were calculated from the data obtained here according to the procedure of *F*-distribution, and the results are shown in Tables 3 and 4. Figs. 1-4 show the effect of each factor graphically. Solid- and dotted lines on the figures represent the cases of significance and no significance, respectively.

All of the four factors were significant in most cases of experiments including two species of wood, five different incubation periods, and two measurements of weight loss and compressive strength. Factor C (fungal species) showed the highest contribution, since *C. globosum* could not degrade both woods at all in all experimental conditions. This apparently evidenced the non-availability of Peptone-Malt Extract

Table 3. Analysis of variance in Experiment I (*Fagus crenata*).

| Factor* | Incubation period (day) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | | 24 | | 36 | | 48 | | 60 | |
| | *F* | ρ % | *F* | ρ % | *F* | ρ % | *F* | ρ % | *F* | ρ % |
| (Based on the loss of weight in wood after decay) | | | | | | | | | | |
| A | 26.55*** | 21.3 | 50.60*** | 31.8 | 300.40*** | 15.3 | 634.44*** | 14.2 | 1386.69*** | 20.5 |
| B | 6.91*** | 4.9 | 9.77*** | 5.6 | 22.49*** | 1.0 | 66.32** | 1.3 | 566.99*** | 8.1 |
| C | 47.19*** | 38.1 | 45.65*** | 28.6 | 754.57*** | 38.7 | 2349.53*** | 53.2 | 1896.59*** | 28.2 |
| D | 2.27** | 1.1 | 4.32** | 2.1 | 727.55*** | 37.3 | 911.66*** | 20.5 | 1732.28*** | 25.8 |
| *e* | | 34.7 | | 31.9 | | 7.7 | | 10.8 | | 17.4 |
| (Based on the compressive strength of wood after decay) | | | | | | | | | | |
| A | 7075** | 6.6 | 107601*** | 24.4 | 130467*** | 20.0 | 205562*** | 19.3 | 571061*** | 32.8 |
| B | 10852*** | 10.9 | 29916*** | 6.1 | 6137*** | 0.4 | 16241** | 1.2 | 110364*** | 5.7 |
| C | 7040** | 6.6 | 73093*** | 16.3 | 261859*** | 40.7 | 624970*** | 59.4 | 403364*** | 23.0 |
| D | 25380*** | 26.9 | 63544*** | 13.8 | 81307*** | 12.2 | 71363*** | 6.5 | 64892*** | 3.0 |
| *e* | | 39.0 | | 39.4 | | 26.7 | | 13.6 | | 35.5 |

* A: Amount of peptone, B: Amount of malt extract, C: Fungal species, D: Size of test block, *e*: Error.

** Significant at 5 % level by *F*-distribution.

*** Significant at 1 % level by *F*-distribution.

ρ: Contribution rate.

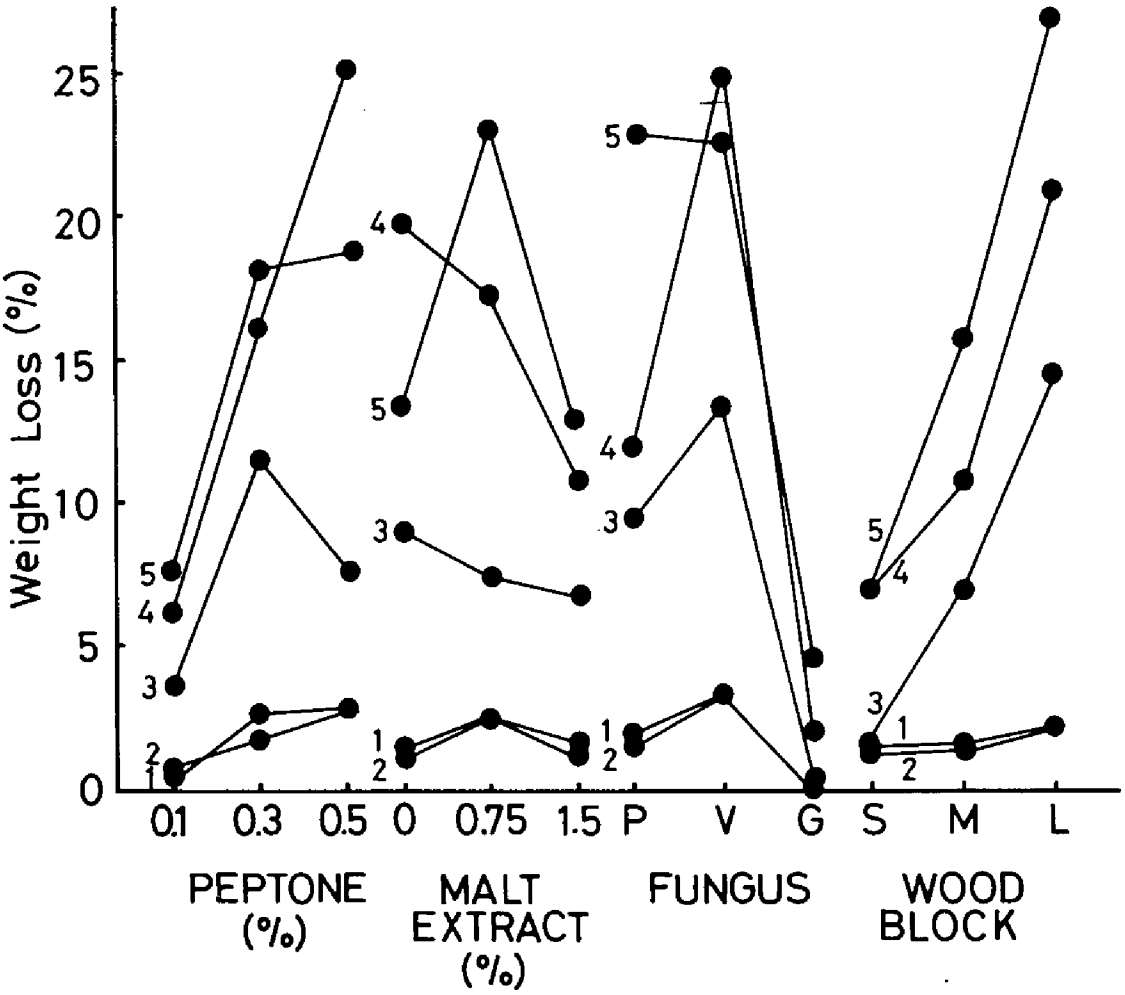



Fig. 1. Effect of factors in Experiment I using *Fagus crenata* (Based on the loss of weight in wood after decay).

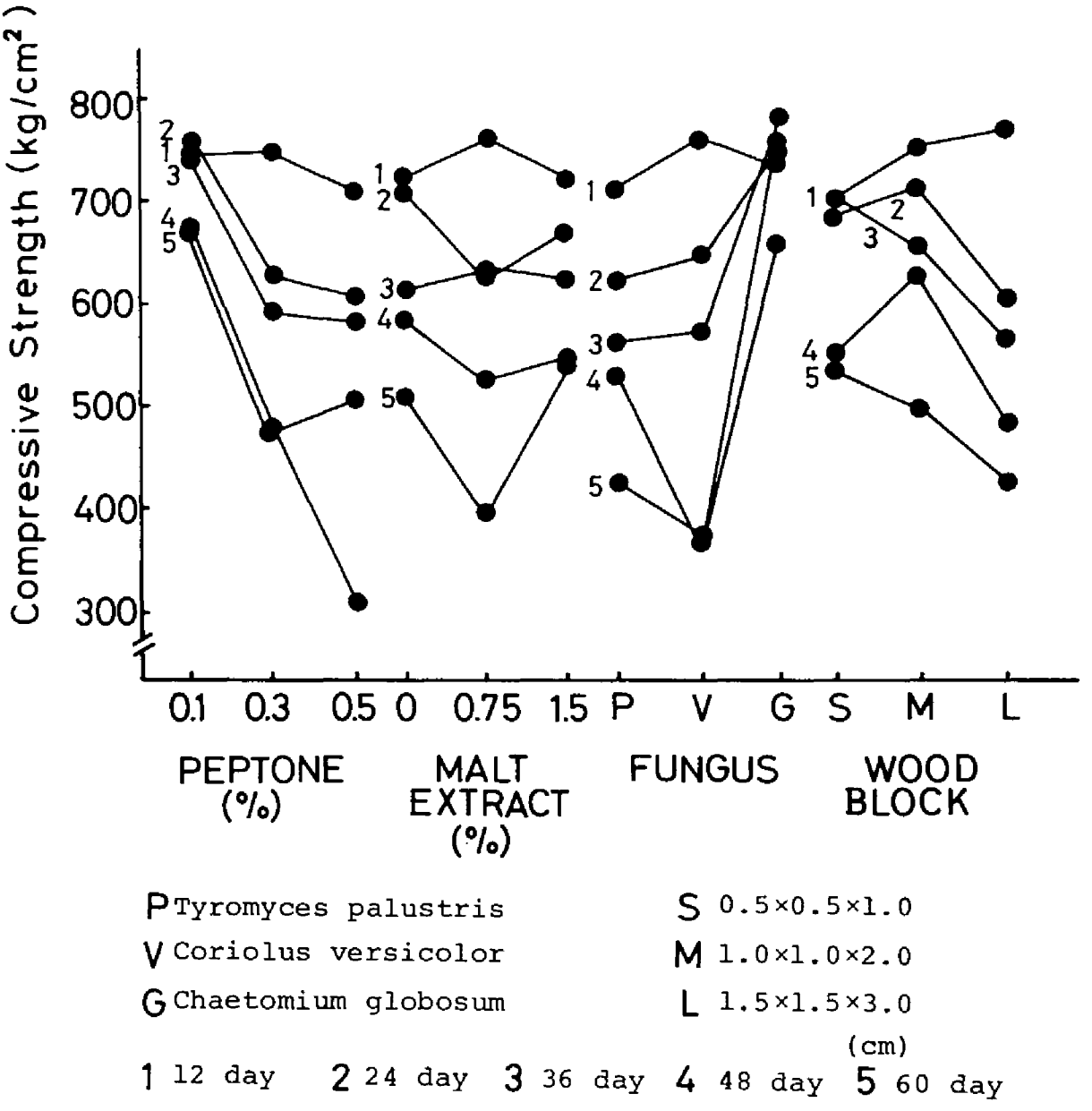



Fig. 2. Effect of factors in Experiment I using *Fagus crenata* (Based on the compressive strength of wood after decay).

Table 4. Analysis of variance in Experiment I (*Cryptomeria japonica*).

| Factor* | Incubation period (day) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | | 24 | | 36 | | 48 | | 60 | |
| | *F* | ρ % | *F* | ρ % | *F* | ρ % | *F* | ρ % | *F* | ρ % |
| (Based on the loss of weight in wood after decay) | | | | | | | | | | |
| A | 111.27*** | 33.7 | 75.83*** | 14.3 | 122.90*** | 10.5 | 106.74*** | 9.0 | 1153.42*** | 26.9 |
| B | 28.56*** | 8.5 | 25.62*** | 4.2 | 76.08*** | 6.3 | 6.83 | 0.0 | 619.12*** | 14.3 |
| C | 95.67*** | 29.1 | 178.26*** | 34.4 | 585.58*** | 51.9 | 421.89*** | 38.6 | 791.52*** | 18.3 |
| D | 66.92*** | 20.2 | 59.61*** | 11.0 | 132.34*** | 11.3 | 34.69*** | 2.2 | 1173.01*** | 27.3 |
| *e* | | 8.5 | | 36.1 | | 20.0 | | 54.2 | | 13.2 |
| (Based on the compressive strength of wood after decay) | | | | | | | | | | |
| A | 6682589*** | 23.0 | 20450*** | 12.4 | 37612*** | 20.1 | 18281*** | 8.7 | 159144*** | 26.1 |
| B | 6687664*** | 23.1 | 3913** | 1.4 | 78 | 0.0 | 15863*** | 7.5 | 68187*** | 11.0 |
| C | 6696827*** | 23.1 | 49129*** | 31.6 | 110894*** | 64.8 | 103964*** | 52.6 | 230650*** | 37.9 |
| D | 6678116*** | 23.0 | 3749** | 1.3 | 15322*** | 6.5 | 3962** | 1.4 | 84545*** | 13.7 |
| *e* | | 7.8 | | 53.3 | | 8.6 | | 29.8 | | 11.3 |

* A: Amount of peptone, B: Amount of malt extract, C: Fungal species, D: Size of test block, *e*: Error.

** Significant at 5 % level by *F*-distribution.

*** Significant at 1 % level by *F*-distribution.

ρ: Contribution rate.

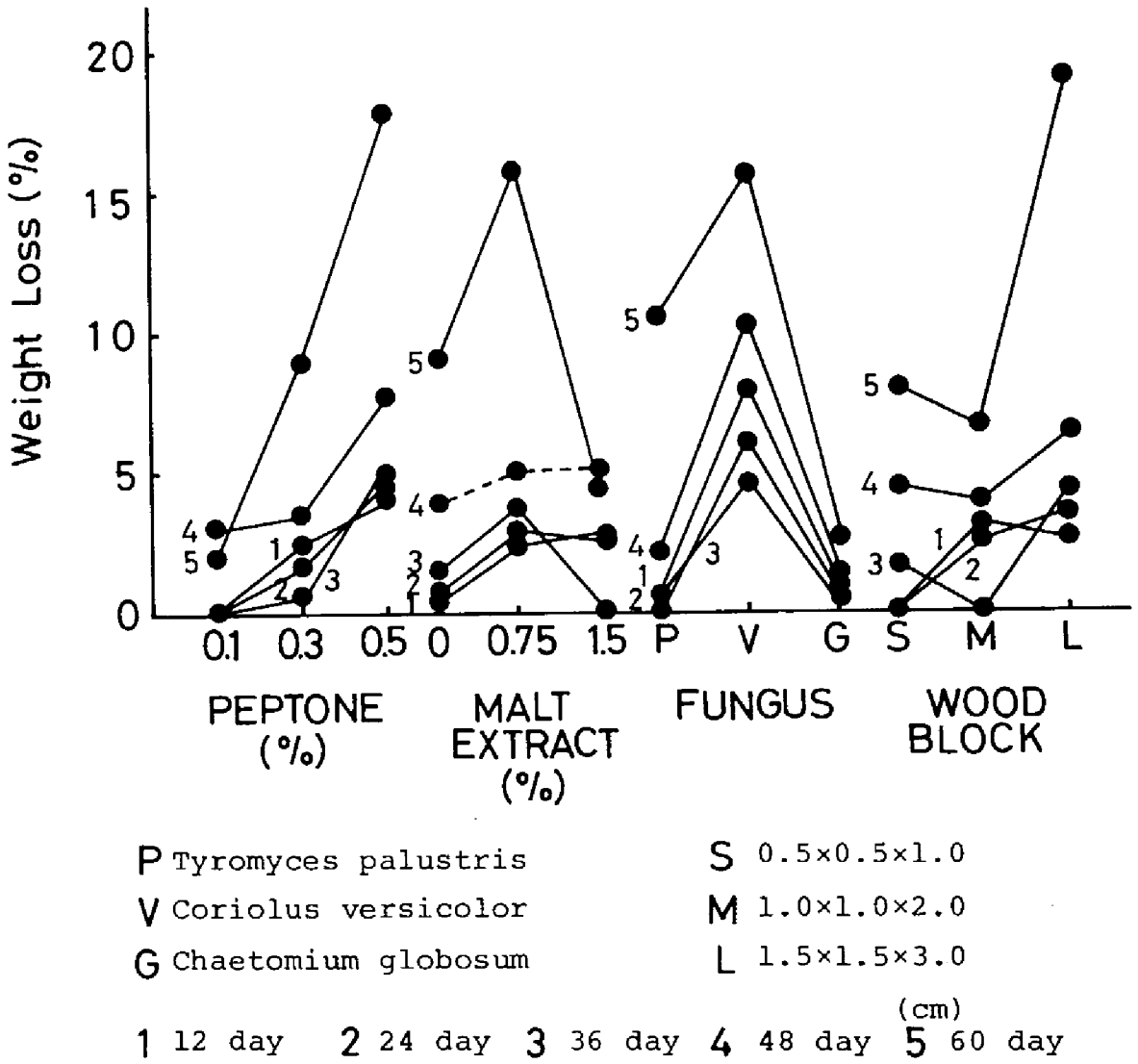



Fig. 3. Effect of factors in Experiment I using *Cryptomeria japonica* (Based on the loss of weight in wood after decay). Dotted line represents the non-significance at 1 and 5 % levels.

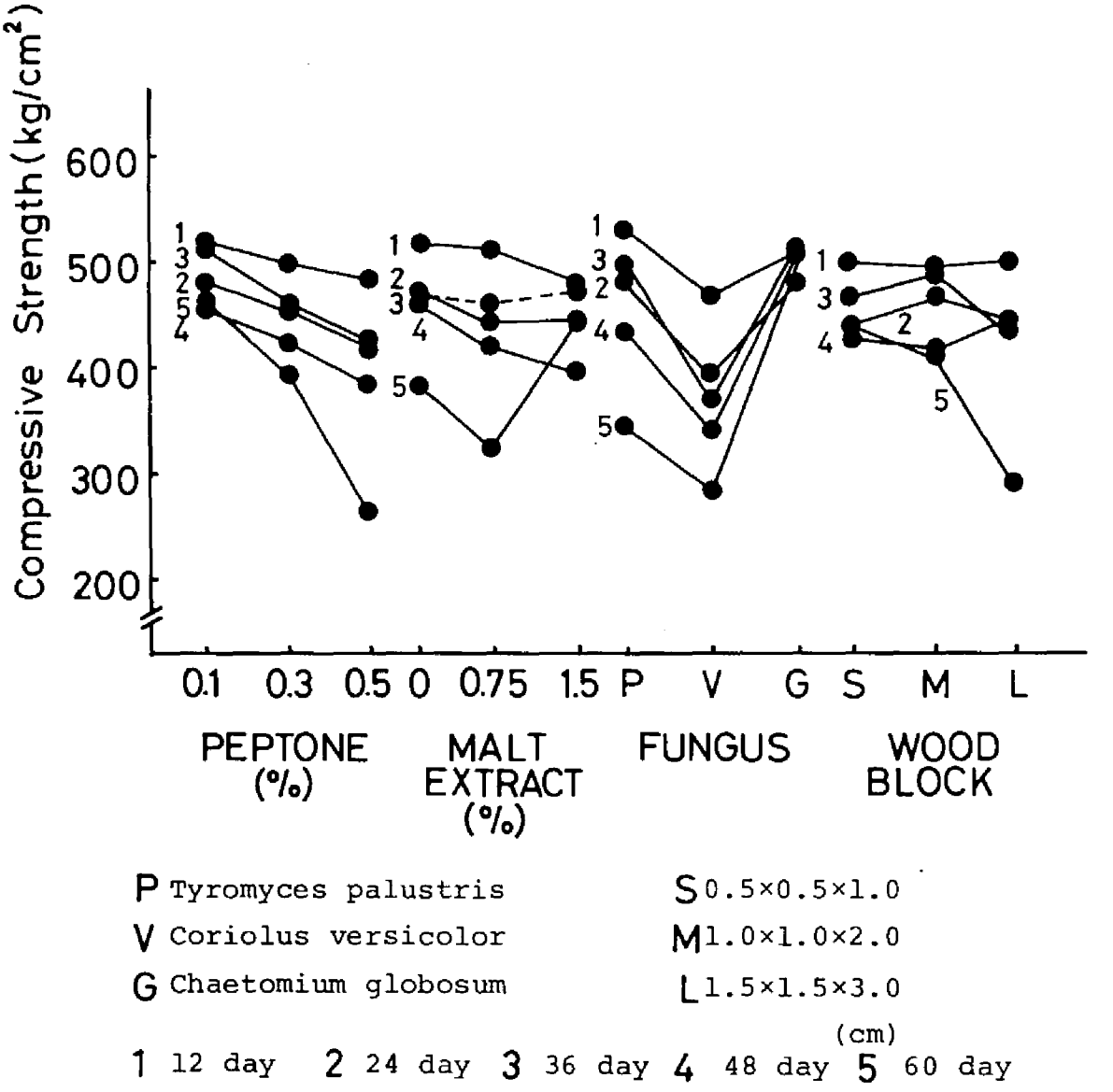



Fig. 4. Effect of factors in Experiment I using *Cryptomeria japonica* (Based on the compressive strength of wood after decay). Dotted line represents the non-significance at 1 and 5 % levels.

Medium to test for *C. globosum*, although fungal growth on the medium was abundant at every experimental condition. The result supports previous proposals[35,43] that higher growth rate of a fungus is not necessarily accompanied with higher growth rate of wood decay. Consequently, significant effects of other factors must be attributed mostly to the activities of the two Basidiomycotina fungi used, and contribution rates of these factors might be greater unless *C. globosum* was used as a test fungus.

Factor A (amount of peptone) was the next to Factor C for the contribution rate. Wood-decaying capacity increased with the amount of peptone. As shown in Table 1, peptone was substantially the sole source of nitrogen in the medium used. This suggests that decrease of the carbon to nitrogen (C:N) ratio brought the increase the rate of decay by the two Basidiomycotina fungi used. Similar result was obtained by Levi and Cowling[82], who investigated the effect of added nitrogen (as casein hydrolysate) on the rate of decay of aspen sapwood by *Polystictus versicolor* (Synonym of *Coriolus versicolor* used in this experiment). In the JIS Z 2119, the amounts of peptone, malt extract and glucose are designated to 0.3 %, 1.5 % and 4.0 %, respectively. However, from the results obtained here, it is concluded that modification of such a composition to decrease C:N ratio is required for obtaining the larger amount of decay.

Factor D (size of test block) was the third in the contribution rate. Higher wood-decaying capacity was observed as the dimentions increased. This was more pronounced in *F. crenata* than in *C. japonica*.

Factor B (amount of malt extract) was the last in order of the contribution. The highest rate of decay was observed at 0.75 % of malt extract.

*C. japonica* was not readily attacked than was *F. crenata*. Poor overall decay was evidenced in *C. japonica* even at 0.3 % level of peptone which permitted the considerable amount of decay of *F. crenata*. This suggests that higher amount of nitrogen should be provided for the incipience of decay of resistant wood.

As to the incubation period, the longer, for example 48- or 60-day, may be required to investigate the effect of each factor more precisely. Difference among the three levels in each factor was not much evidenced for the shorter periods of incubation.

Significance of factor determined by the compressive strength was approximately the same to that by the loss of weight. In *F. crenata*, however, contribution rate of error by the former was always slightly greater than that by the latter. This means that some factors other than the four factors may contribute more actively at the determination of the compressive strength. They seem to be involved in the condition of measurement, the uniformity of test blocks and the location of destruction in decayed blocks. However, the advantage of strength determination, as means of evaluating the wood-decaying capacity, was never minimized by these unknown factors.

## 1-2 Experiment II using Enriched Abrams's Medium[114)]

### Materials and Methods

Four experimental factors with three levels each were dealt in the Experiment II using enriched Abrams's Medium. These factors are: size of test block, wood species, amount of ammonium nitrate and kind of sugar. Ammonium nitrate and sugar are the sole sources of nitrogen and carbon, respectively. Original Abrams's Medium[1)] contains 3 g of $NH_4NO_3$, 2 g of $K_2HPO_4$, 2.5 g of $KH_2PO_4$ and 2 g of $MgSO_4 \cdot 7H_2O$ per litre of water. This synthetic medium was used by Savory[103)] together with several media for the isolation of fungi from cooling towers. He showed that *C. globosum* caused the highest weight loss of beech blocks on Abrams's agar. Nitrates are known to be excellent sources of nitrogen for many fungi, although inability to utilize nitrates is especially common in some groups, e.g., the higher Basidiomycotina, the Saplolegniales and Blastocladiales of Mastigomycotina, etc[30)]. However, the effect of nitrate concentration in the medium on the rate of decay has been little studied.

As described previously, mycelial growth on the whole surface of medium prior to subjecting test blocks to fungal attack has been recommended for the successful performance of decay tests. Addition of sugar as carbon source is essential for desirable abundant mycelial growth.

Test blocks were prepared from the sapwoods of *Fagus crenata* Blume, *Shorea* sp. commonly called "red lauan", and *Pinus densiflora* Sieb.et Zucc. The sizes of test block are the same as those in Experiment I. Concentrations of ammonium nitrate are: 0.1, 0.3 and 0.5(%). Kinds of suga

are: D-glucose, D-xylose and D-mannose. Concentration of each sugar is equally 2.0 %.

The nine experiments were laid out according to the orthogonal table $L_9(3^4)$ as shown in Table 5.

All the procedures involved in the decay test and the determination of compressive strength are the same as those of Experiment I.

Table 5. Lay out of Experiment II using Enriched Abrams's Medium.

| Factor / No. | A $NH_4NO_3$ (%) | B Carbon source | C Wood* | D Test block** |
|---|---|---|---|---|
| 1 | 0.1 | glucose | P | small |
| 2 | 0.1 | xylose | F | middle |
| 3 | 0.1 | mannose | L | large |
| 4 | 0.3 | glucose | F | large |
| 5 | 0.3 | xylose | L | small |
| 6 | 0.3 | mannose | P | middle |
| 7 | 0.5 | glucose | L | middle |
| 8 | 0.5 | xylose | P | large |
| 9 | 0.5 | mannose | F | small |

* P: *Pinus densiflora*, F: *Fagus crenata*, L: *Shorea* sp.

** small: 0.5 × 0.5 × 1.0, middle: 1.0 × 1.0 × 2.0, large: 1.5 × 1.5 × 3.0 (cm).

## Results and Discussion

Analyses of variances were calculated similarly to Experiment I, and the results are shown in Table 6. The effect of each factor is shown in Figs. 5 and 6.

All of the four factors were significant at every incubation period

Table 6. Analysis of variance in Experiment II.

| Factor* | Incubation period (day) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | | 24 | | 36 | | 48 | | 60 | |
| | *F* | ρ % | *F* | ρ % | *F* | ρ % | *F* | ρ % | *F* | ρ % |
| (Based on the loss of weight in wood after decay) | | | | | | | | | | |
| A | 2.05 | 0.8 | 25.98*** | 1.8 | 17.01** | 0.8 | 207.13*** | 7.3 | 161.69*** | 6.2 |
| B | 5.65** | 3.1 | 2.13 | 0.0 | 1.98 | 0.0 | 22.59*** | 0.7 | 6.49*** | 0.1 |
| C | 80.90*** | 52.7 | 826.25*** | 70.1 | 1021.39*** | 64.9 | 2021.84*** | 74.3 | 2100.36*** | 82.1 |
| D | 27.30*** | 17.4 | 151.23*** | 12.6 | 357.58*** | 22.5 | 340.48*** | 12.1 | 160.42*** | 6.2 |
| *e* | | 26.0 | | 15.5 | | 13.8 | | 5.6 | | 5.4 |
| (Based on the compressive strength of wood after decay) | | | | | | | | | | |
| A | 13207*** | 1.4 | 8001 | 1.8 | 4001 | 0.4 | 3029 | 0.1 | 31014** | 3.3 |
| B | 62675*** | 10.7 | 64621*** | 15.5 | 43021*** | 7.3 | 4440 | 0.4 | 54986*** | 5.8 |
| C | 41725*** | 6.7 | 123855*** | 29.8 | 363582*** | 64.6 | 367229*** | 63.5 | 631352*** | 67.0 |
| D | 18392*** | 2.3 | 24660*** | 5.8 | 72897*** | 12.7 | 47357*** | 7.8 | 99388*** | 10.5 |
| *e* | | 78.9 | | 47.1 | | 15.0 | | 28.2 | | 13.4 |

* A: Amount of ammonium nitrate, B: Kind of sugar, C: Wood species, D: Size of test block, *e*: Error.

** Significant at 5 % level by *F*-distribution.

*** Significant at 1 % level by *F*-distribution.

ρ: Contribution rate.

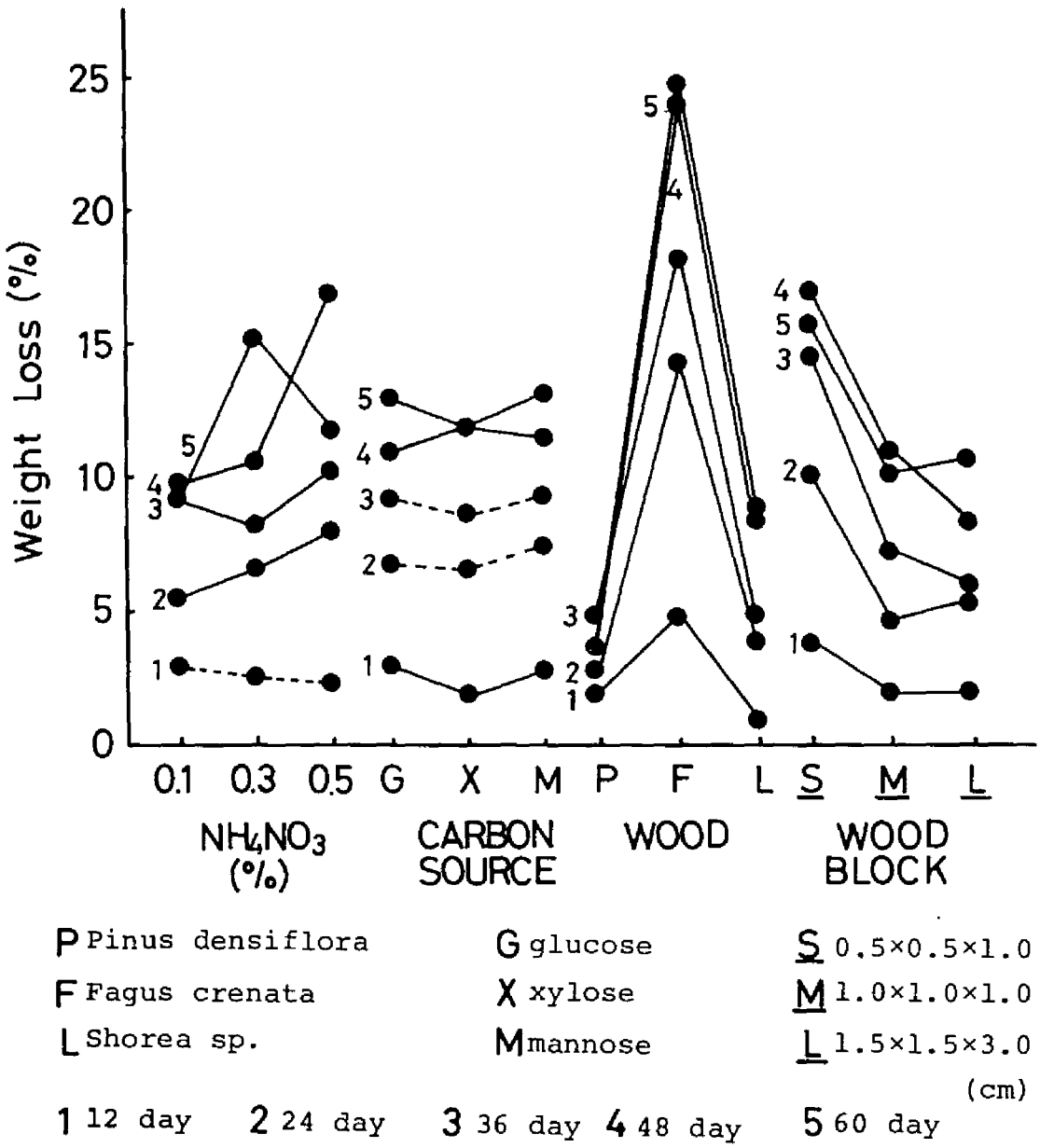



Fig. 5. Effect of factors in Experiment II (based on the loss of weight in wood after decay). Dotted line represents the non-significance at 1 and 5 % levels.

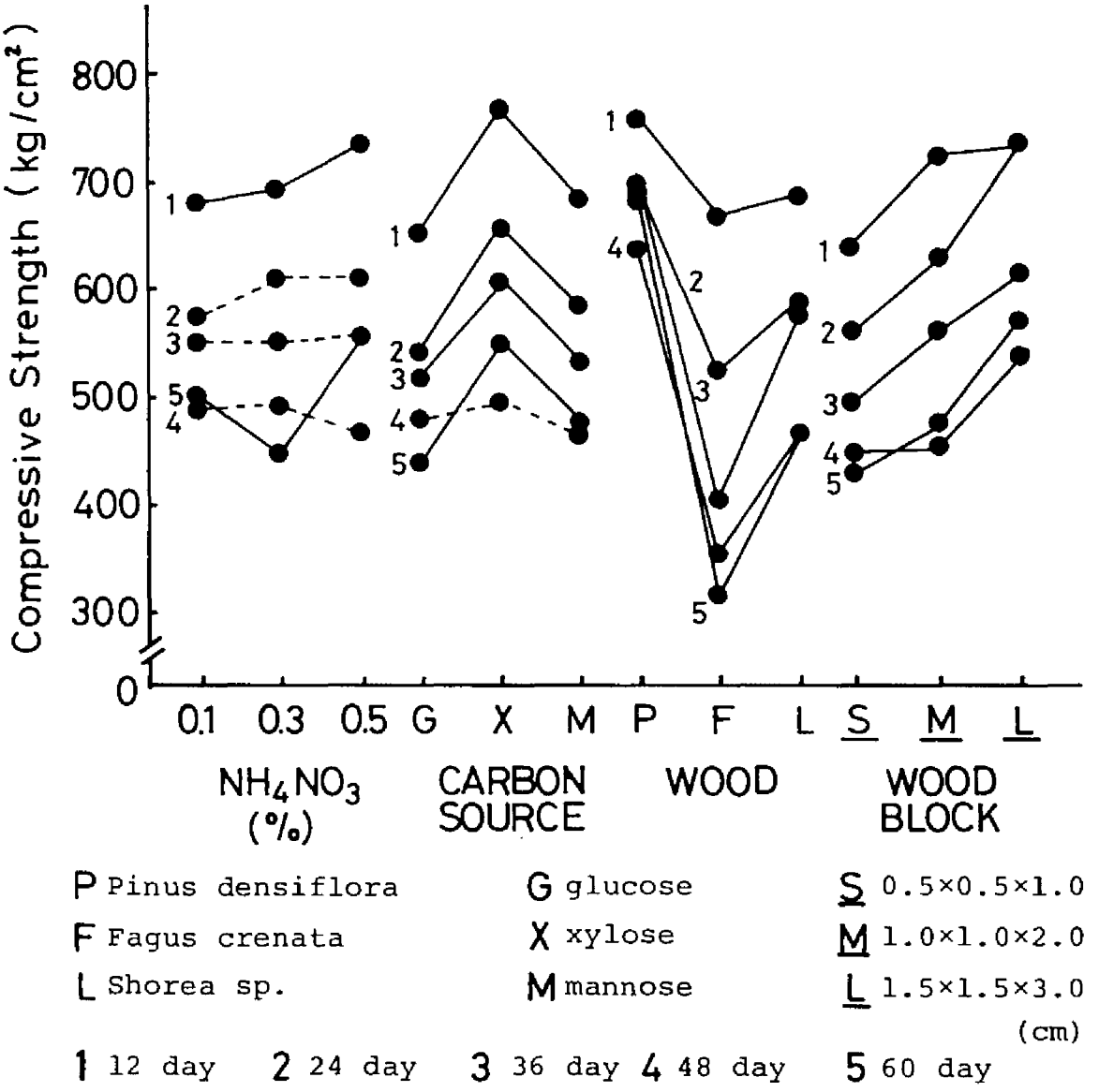



Fig. 6. Effect of factors in Experiment II (Based on the compressive strength of wood after decay). Dotted line represents the non-significance at 1 and 5 % levels.

in either weight loss or compressive strength. Factor C (wood species) showed the highest contribution rate, since *C. globosum* could not attack substantially the test blocks of *P. densiflora* and *Shorea* sp. The contribution rates of other factors might be greater if more susceptible species are used. The effects of Factor A (amount of ammonium nitrate) and Factor B (kind of sugar) were not so much obvious. However, higher amount of wood decay was obtained at 0.3 or 0.5 % of ammonium nitrate and on the medium containing glucose or mannose. The compressive strength of test blocks remained at higher level on xylose-containing medium.

Factor D (size of test block) was the next to Factor C for the order of the contribution rate. "Small" blocks were attacked more severely than others. The effect of size was remarkably pronounced in the compressive strength. Similar results have been published recently by Kerner-Gang[68], who investigated several factors influencing soft rot testing in the vermiculite burial method. Decomposition rates of preservatives-treated beech blocks were much higher for the smaller ones.

The contribution rate of error in the compressive strength was always greater than that in the loss of weight.

The results obtained here showed that forty-eight or sixty day's incubation was required to investigate the preciser effect of each factor.

## 1-3 Summary

To investigate the cultural conditions which allow the higher wood-decaying capacity of soft rot fungus, *Chaetomium globosum* Kunze, the effects of variations in amounts of carbon- and nitrogen sources, kind of carbon source and size of test wood block were discussed, using the Peptone-Malt Extract Medium and the enriched Abrams's Medium. Experiments were laid out according to the orthogonal table in the "design of experiment". Effects of these variations were estimated by the statistical analysis of variance.

Non-availability of the Peptone-Malt Extract Medium for testing *C. globosum* was undoubtedly evidenced. Modification of the medium to decrease C:N ratio resulted in the larger amount of decay by Basidiomycotina. Availability of the Abrams's Medium for *C. globosum* was confirmed here again. Addition of 0.3 % of ammonium nitrate was superior to 0.1 % and equal to 0.5 %. D-Xylose as sole source of carbon was slightly inferior to D-glucose and D-mannose. Smaller blocks were attacked more severely than larger blocks by *C. globosum*, while the reverse result was obtained for the Basidiomycotina used. Forty-eight or sixty day's incubation was required to estimate the preciser effect of each factor and to yield more than 20 % of weight loss of beech blocks by *C. globosum*.

# CHAPTER 2 EFFECT OF CHAETOMIUM GLOBOSUM ON THE PHYSICAL- AND CHEMICAL PROPERTIES OF WOOD

As described in Chapter 1, non-availability of the Peptone-Malt Extract Medium to decay test for *C. globosum* was strongly evidenced. This suggests that wood-decaying capacity of this fungus is not exhibited in a nutritional condition which is favorable to brown rot- and white rot fungi. However, detailed study on chemical and physical effects of soft rot fungi has not appeared yet in contrast with other wood-decaying fungi. A soft rot fungus, *C. globosum*, resembles to brown rot fungi on the carbohydrates depletion with little lignin attack, while that is more analogous to white rot fungi on the gradual increase in alkali solubility of rotted wood[105]. Levi and Preston[81] observed that DP (degree of polymerization) of the holocellulose during the attack of beech wood by this fungus initially increased and then decreased gradually. This mode of attack seems to differ from that of either brown rot fungi or white rot fungi, which cause a rapid loss in DP of the total cellulose[33], or only a gradual loss in DP[33]. Cartwright et al.[24] found long ago that brown rot fungi cause a more rapid drop in strength properties than do white rot fungi. Although the reduction in impact bending strength was determined for soft-rotted wood by a few investigators[4,85], degrees of reduction in other strengths have not been studied yet, and no comparison with those for brown-rotted- and white-rotted woods has been made.

To obtain information on the characteristics of wood decay by soft rot fungi, (1) ability of *C. globosum* to reduce the strengths of wood

has been compared with that of white rot- and brown rot fungi, and (2) infrared spectral changes in wood constituents during the attack by *C. globosum* and (3) carbon nutrition of this fungus are investigated.

## 2-1 Changes in strength properties of wood on decay[115)]

### Materials and Methods

*Wood blocks*

Wood blocks were prepared from the sapwoods of *Fagus crenata* Blume and *Cryptomeria japonica* D. Don. The sizes of block are as follows:

(i) for the compressive strength parallel to the grain: 1.5 ($t$) × 1.5 ($r$) × 3.0 ($l$) (cm),

(ii) for the compressive strength perpendicular to the grain: 1.5 ($t$) × 1.5 ($l$) × 3.0 ($r$) (cm),

(iii) for the static bending strength: 1.0 ($t$) × 1.0 ($r$) × 14.0 ($l$) (cm),

(iv) for the tensile strength parallel to the grain: illustrated in Fig. 7.

For enhancing fungal attack at the central part, blocks for bending- and tensile strengths were sealed with paraffin and paraffin-films on their surfaces with the exception of central parts - 2.5 cm length each from the center and the portion (C) in Fig. 7, respectively.

*Test fungi*

In addition to a soft rot fungus, *Chaetomium globosum* Kunze (IAM 8059), a white rot fungus, *Coriolus versicolor* Quél. (FES 1030), and a brown rot fungus, *Tyromyces palustris* Murr. (FES 0507), were used as

test fungi.

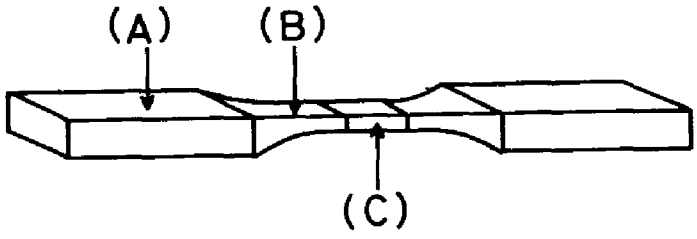



Fig. 7. Size of the tensile test wood block exposed to the fungal attack.
(A): Cross sectional area 1.5 × 0.85 $cm^2$, length 5.0 cm,
(B): Radius of curvature, radial 5.7 cm, tangential 3.5 cm,
(C): Cross sectional area 0.66 × 0.33 $cm^2$, length 1.65 cm.

*Decay test*

The decay test was carried out by the sand-block method. Cylindrical glass bottles (described in Chapter 1), containing 350 g of quartz sand (ca. 30 mesh) and 120 ml of nutrient solution, were screwed with metal caps. Rectangular glass bottles (illustrated in Fig. 8), containing 500 g of the sand and 170 ml of the solution, were capped with four-layers of alminium foils. The former was used for the decay of blocks for compressive strength, and the latter was used for bending- and tensile strengths. These bottles were autoclaved and inoculated with the test fungi which were allowed to cover the surface of the medium before wood blocks were inserted. The composition of the nutrient solution is as follows:

for the decay test using *C. globosum*,

$NH_4NO_3$ 3.0 g, $KH_2PO_4$ 2.5 g, $K_2HPO_4$ 2.0 g, $MgSO_4 \cdot 7H_2O$ 2.0 g, glucose 30.0 g and distilled water 1000 ml.

for the decay test using *C. versicolor* and *T. palustris*,

$KH_2PO_4$ 3.0 g, $MgSO_4 \cdot 7H_2O$ 2.0 g, peptone 5.0 g, malt extract 20.0 g, glucose 50.0 g and distilled water 1000 ml.

The weighed and measured blocks were sterilized by fumigation with propylene oxide, soaked in sterilized distilled water and exposed to test fungi. Incubation periods were varied with the dimensions of blocks. The temperature was maintained at 28°C throughout five different incubation periods of 10-, 25-, 45-, 70- and 100-days for compressive strengths, and of 10-, 20-, 30-, 45- and 60-days for bending- and tensile strengths. Ten blocks in three bottles were used in each series of decay tests. The decayed blocks were cleaned of mycelium and dried to constant weight in an oven at 65°C. After determination of the weight, specific gravity and dimensions, the decayed blocks were submitted to the tests of strengths.

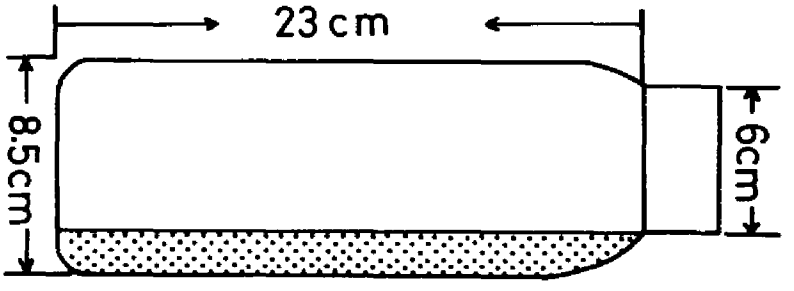



Fig. 8. Size of the rectangular glass bottle used in the decay test.

*Measurement of strength*

Compressive- and tensile strengths were determined by a Shimadzu REH-10 multi-purpose testing machine. Determination of bending strength was made under a static center loading by a Swedish bending testing machine. Modulus of elasticity in bending was also calculated from the deflection value during the loading.

## Results and Discussion

*Compressive strength parallel to the grain*

Results are shown in Figs. 9 and 10. When *F. crenata* was exposed to *T. palustris*, reduction of the strength was rapid during the decrease of specific gravity to the value of 0.36, and then gradual. The strength for the brown-rotted beech was expressed mathematically by the equation ($P = 2250d - 650$, $d \geqq 0.36$), where $P$ and $d$ are the strength and the specific gravity, respectively. In the soft-rotted beech, decay was concentrated largely at the surface part of block and did not proceed into the inner portion. However, similar equation ($P = 2250d - 670$) was obtained within the limited range of specific gravity over 0.40. On the other hand, the strength for the white-rotted beech did not reduce rapidly and the equation ($P = 2000d - 670$, $d \geqq 0.40$) was applicable. In the case of *C. japonica* exposed to *C. globosum*, neither loss of weight nor reduction in strength was obvious. The reduction in strength of attacked wood of *C. japonica* was evidently rapider for *T. palustris* ($P = 4100d - 980$, $d \geqq 0.24$) than for *C. versicolor*.

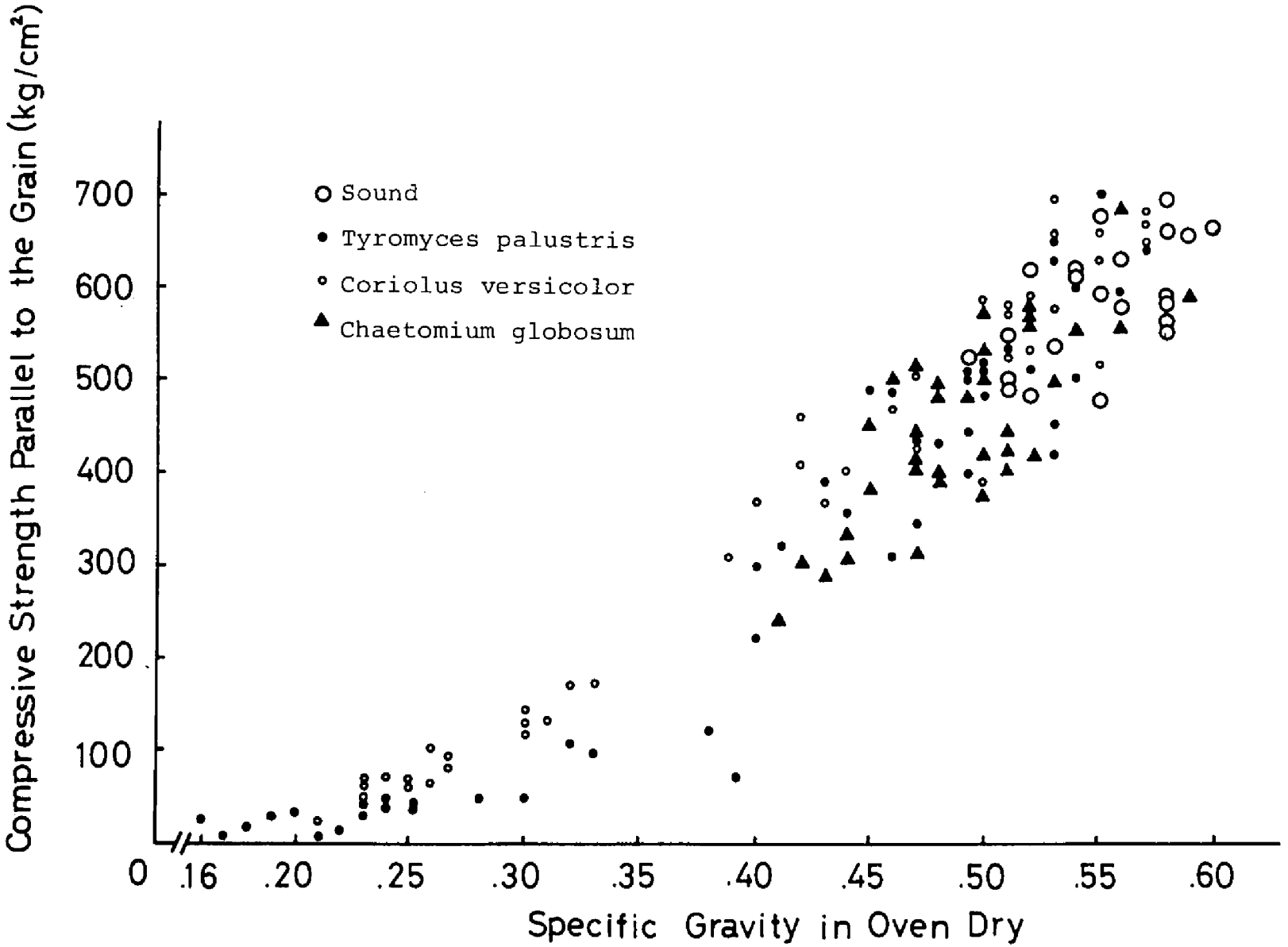



Fig. 9. Reduction in compressive strength (*f*-//) of wood of *Fagus crenata* exposed to the test fungi.

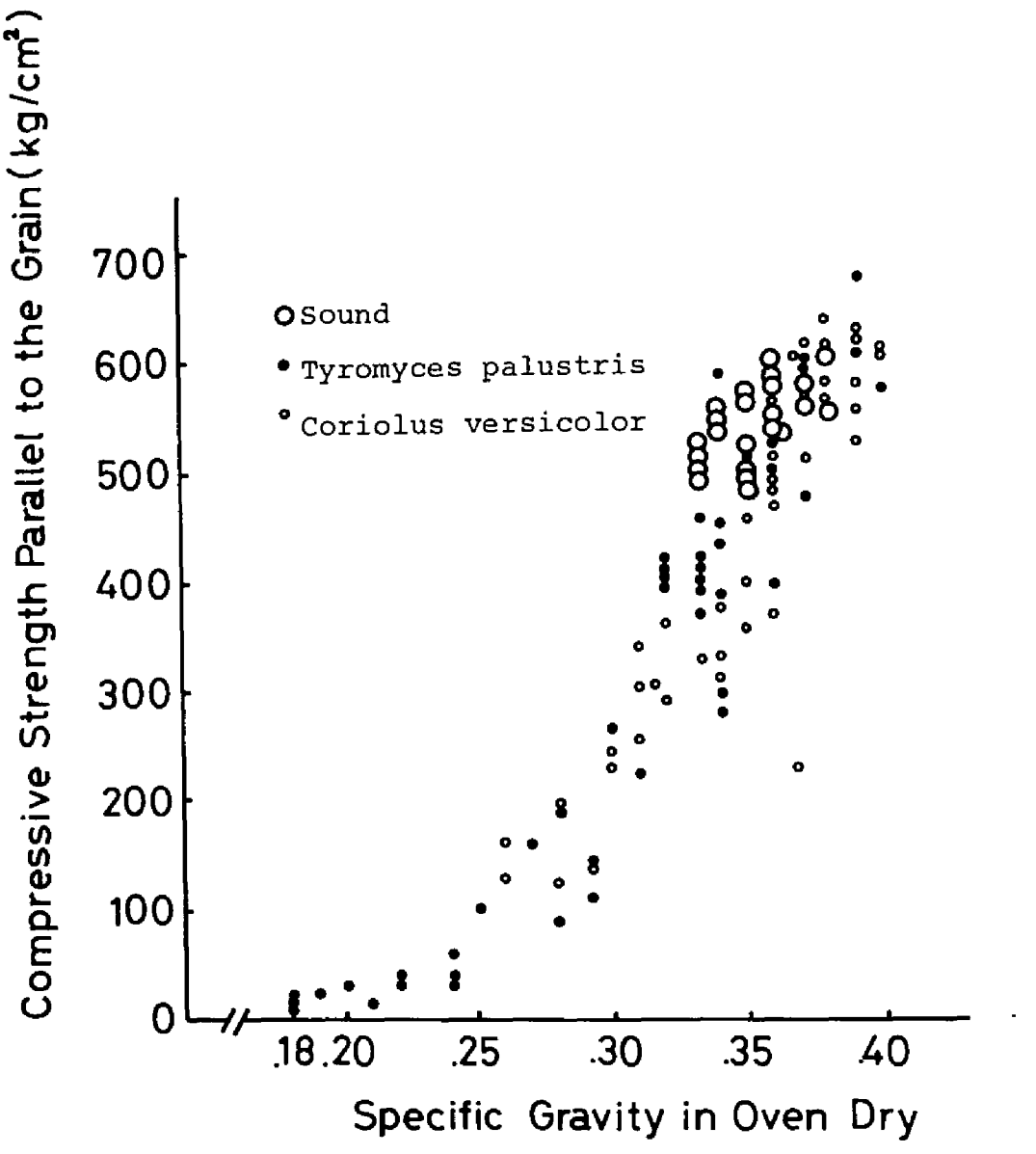



Fig. 10. Reduction in compressive strength (*f*-//) of wood of *Cryptomeria japonica* exposed to the test fungi.

*Compressive strength perpendicular to the grain*

Results are shown in Figs. 11 and 12. In *F. crenata* exposed to *C. globosum*, the compressive strength perpendicular to the grain reduced more rapidly than that parallel to the grain. The reduction in the

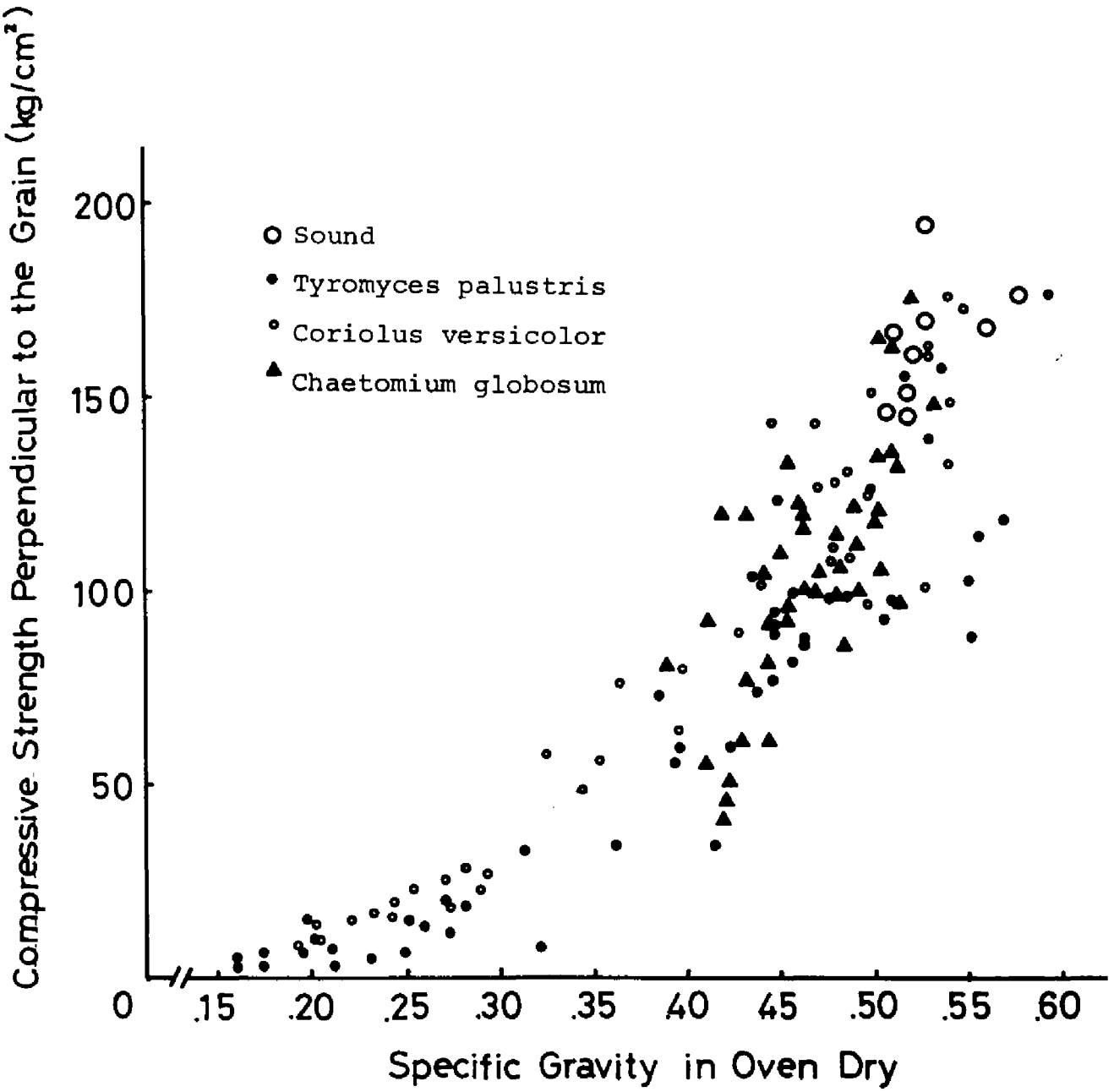



Fig. 11. Reduction in compressive strength ($f$-$\perp$) of wood of *Fagus crenata* exposed to the test fungi.

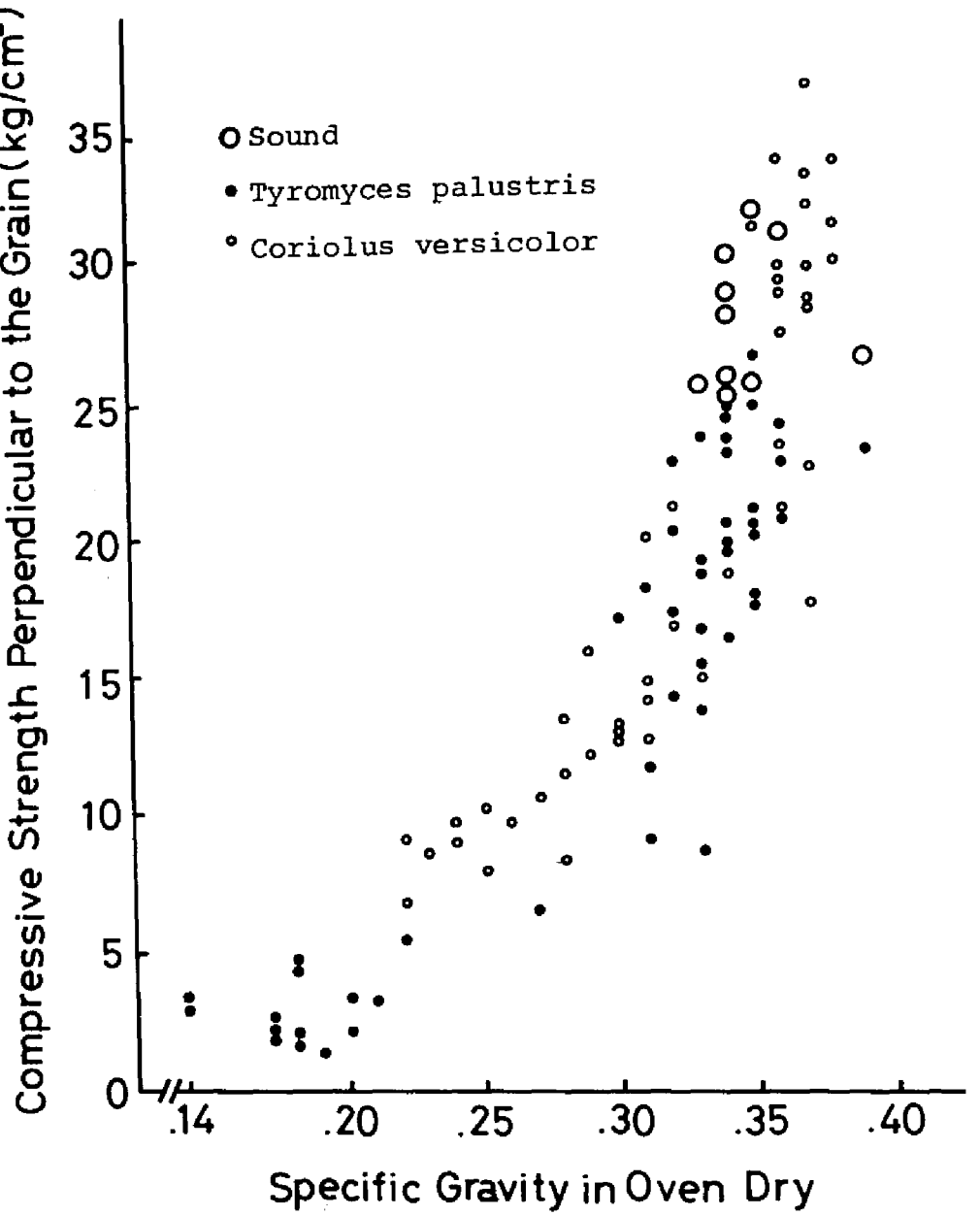



Fig. 12. Reduction in compressive strength ($f$-$\perp$) of wood of *Cryptomeria japonica* exposed the test fungi.

strength for the brown-rotted beech preceded similarly that for the white-rotted one. The former was expressed by the equation ( $P = 550d$ -

170, $d \geqq 0.30$), and the latter by the equation ($P = 500d - 125$, $d \geqq 0.30$). The reduction in the strength for the wood of *C. japonica* was rapider than for *F. crenata*.

*Static bending strength*

Results are shown in Figs. 13-16. Relation between the bending strength and the modulus of elasticity is shown in Figs. 14 and 16. Relation between the strength and the specific gravity is shown in Figs. 13 and 15. The specific gravity was not calculated from the weight and the volume of the whole block but from those of the central portion where decay was concentrated. The reductions in the strength for both the brown-rotted woods were rapider than those for the white-rotted and the soft-rotted ones in both wood species. The reduction for the soft-rotted wood of *F. crenata* preceded that for the white-rotted when the specific gravity reduced to ca 0.45, although approximately the same pattern at the greater specific gravity was observed for both types of decay. The modulus of elasticity decreased in all the cases as in the reduction in strength. The reductions in strength for the brown-rotted and soft-rotted beech blocks were especially rapid within the range between 40 and 50 of the modulus of elasticity. The rate of reduction in the strength was higher for the wood of *C. japonica* than that for *F. crenata*.

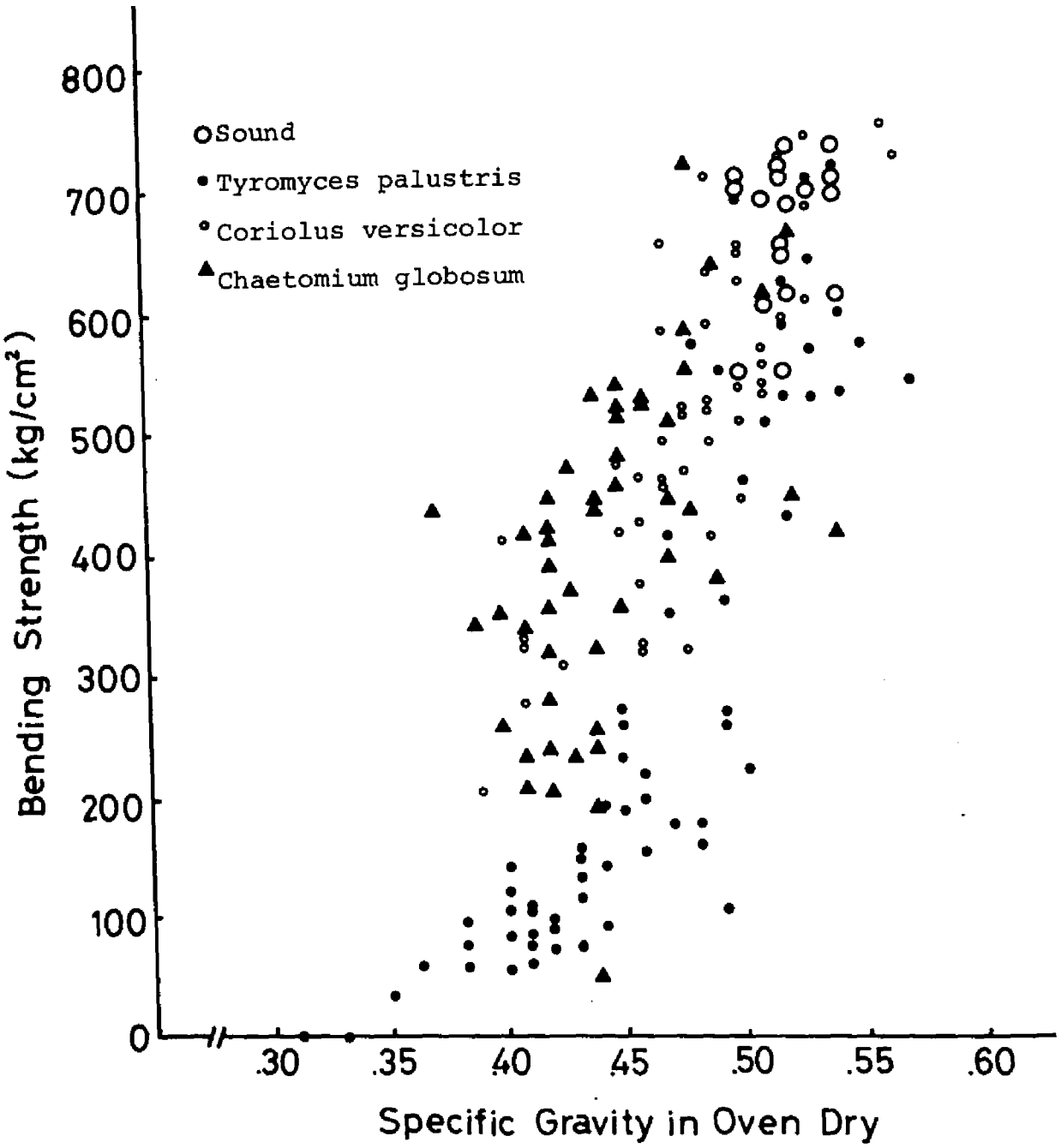



Fig. 13. Reduction in bending strength of wood of *Fagus crenata* exposed to the test fungi.

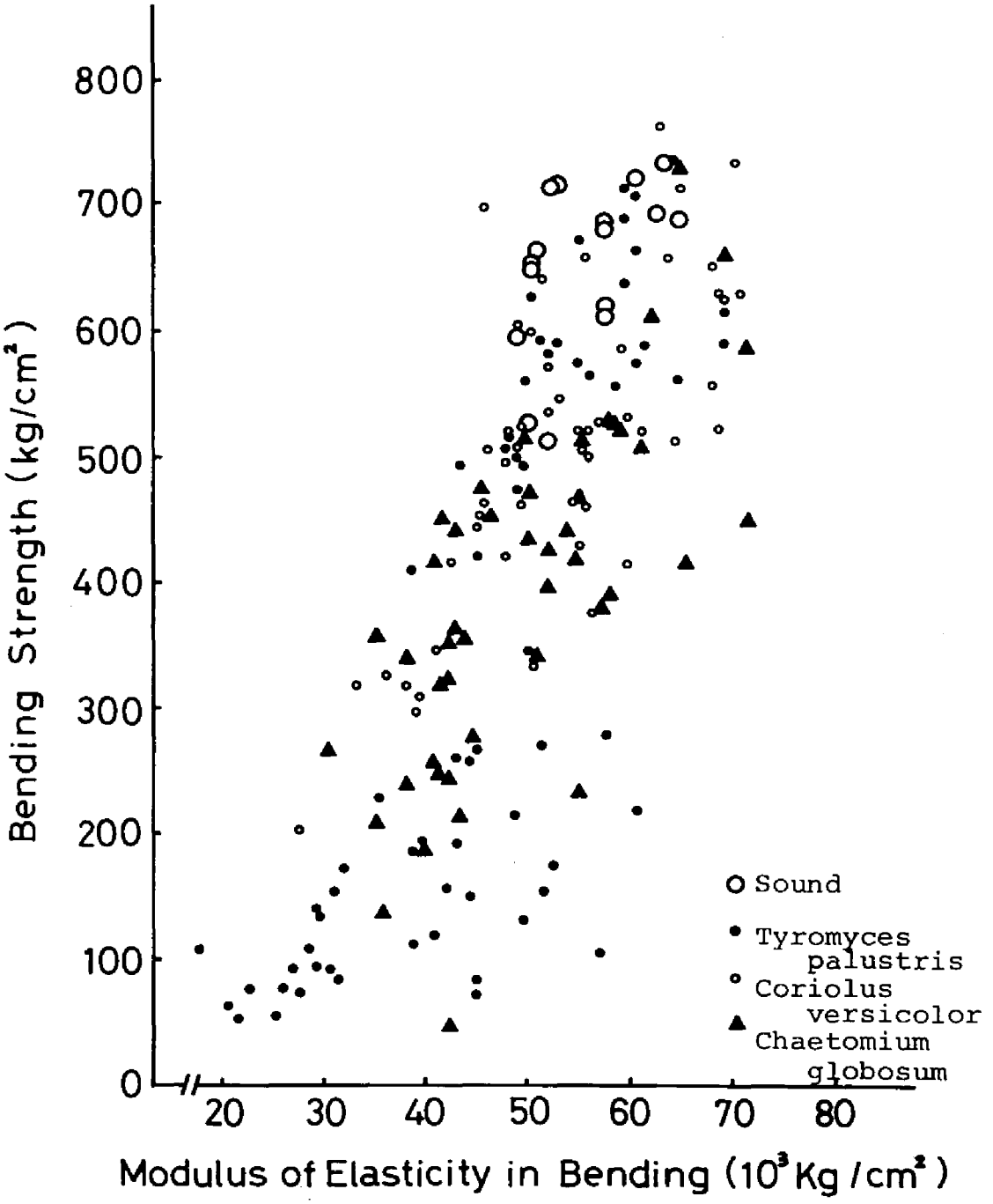



Fig. 14. Relation between strength and modulus of elasticity in bending in wood of *Fagus crenata* exposed to the test fungi.

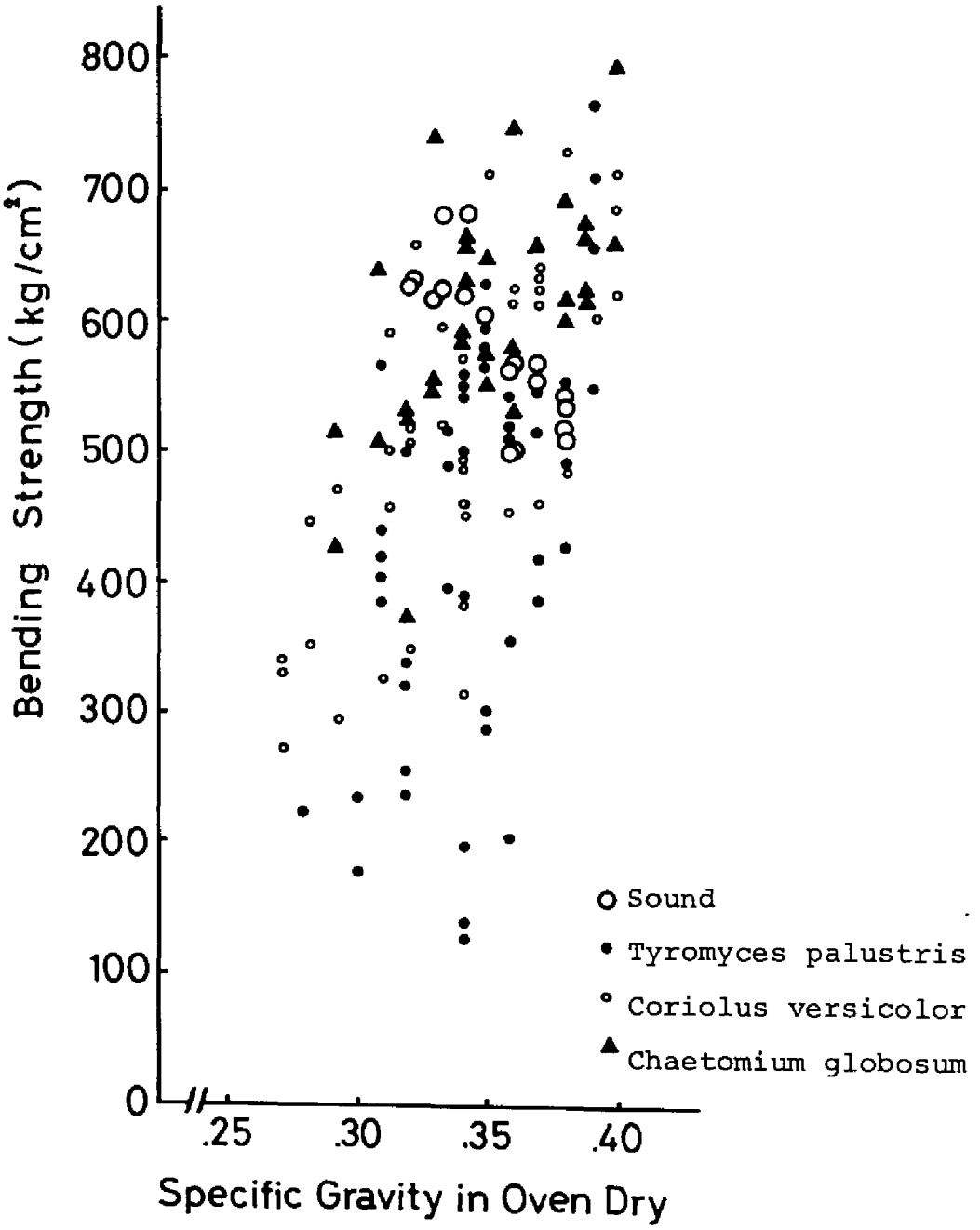



Fig. 15. Reduction in bending strength of wood of *Cryptomeria japonica* exposed to the test fungi.

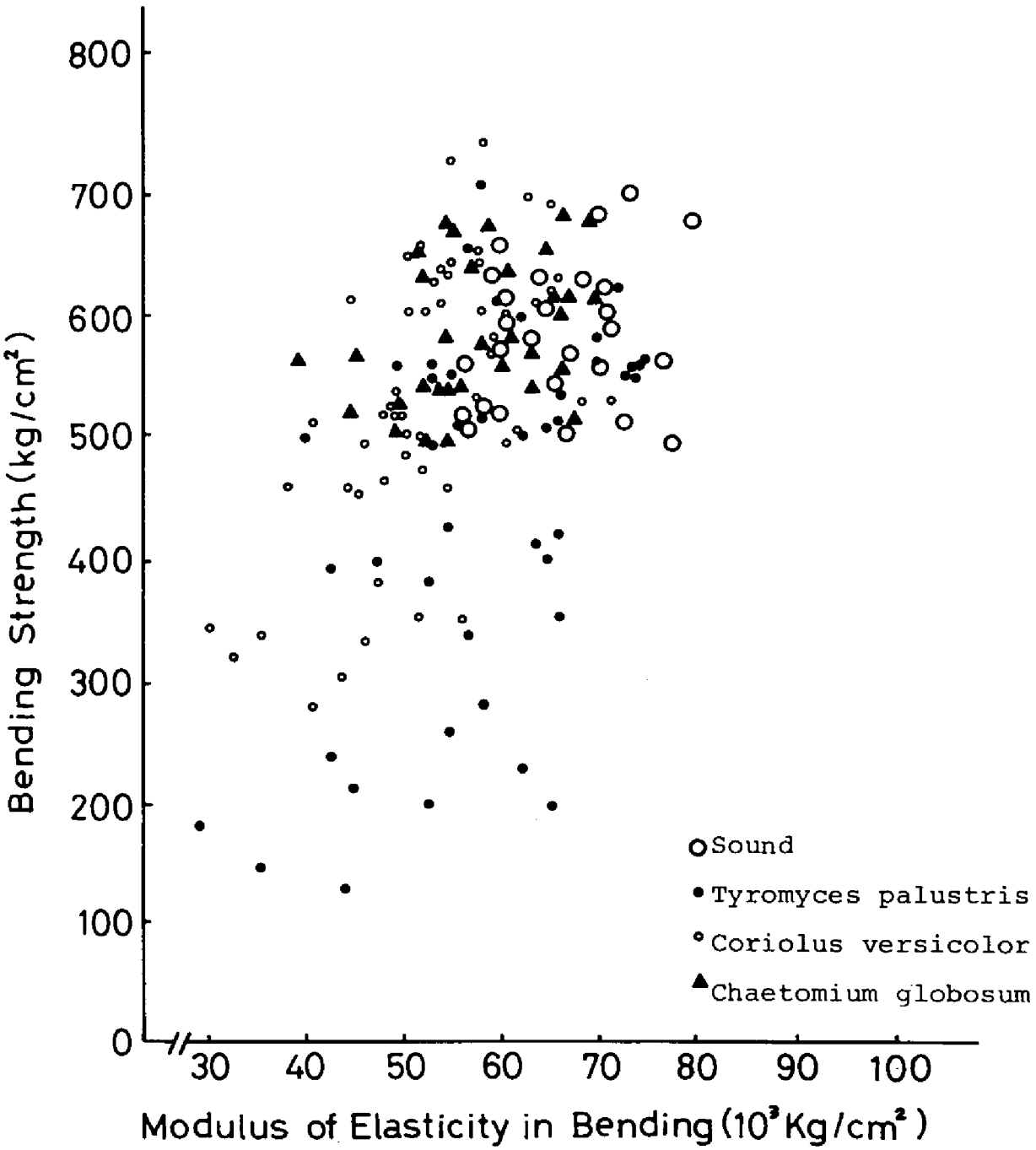



Fig. 16. Relation between strength and modulus of elasticity in bending in wood of *Cryptomeria japonica* exposed to the test fungi.

*Tensile strength parallel to the grain*

Results are shown in Figs. 17 and 18. Determination of the specific gravity was similarly made on the central portion of block. Clear patterns were not obtained due to the greater variations among data and to the lower rate of decay. However, the reductions in the strength for the brown-rotted woods seemed to still precede those for other woods. The reduction was evidently detected even in the wood of *C. japonica* exposed to *C. globosum*.

Changes in strength properties of wood caused by decay have been actively studied since Cartwright et al's 1931 publication[24]. In all early studies, strengths versus incubation periods or losses of weight in whole wood blocks were plotted on graphs. As described in Chapter 1, size of wood blocks usually influences the rate of decay. Size of blocks should be varied with kind of strength determination. In the determination of bending- and tensile strengths, the rate of decay of the portion where loading and stress are concentrated greatly influences the results. On the long and slender block used for the test of bending strength, a uniform rate of decay is never expected throughout the whole length of block. Furthermore, on the block for tensile strength, a precise determination is not made when the ends of block are degraded by fungal attack. In these cases, the reduction in strength can not be compared exactly with the loss of weight in whole block. To enhance the decay at the central portion of block, blocks for the bending- and tensile strengths were sealed with paraffin and paraffin films leaving the cen-

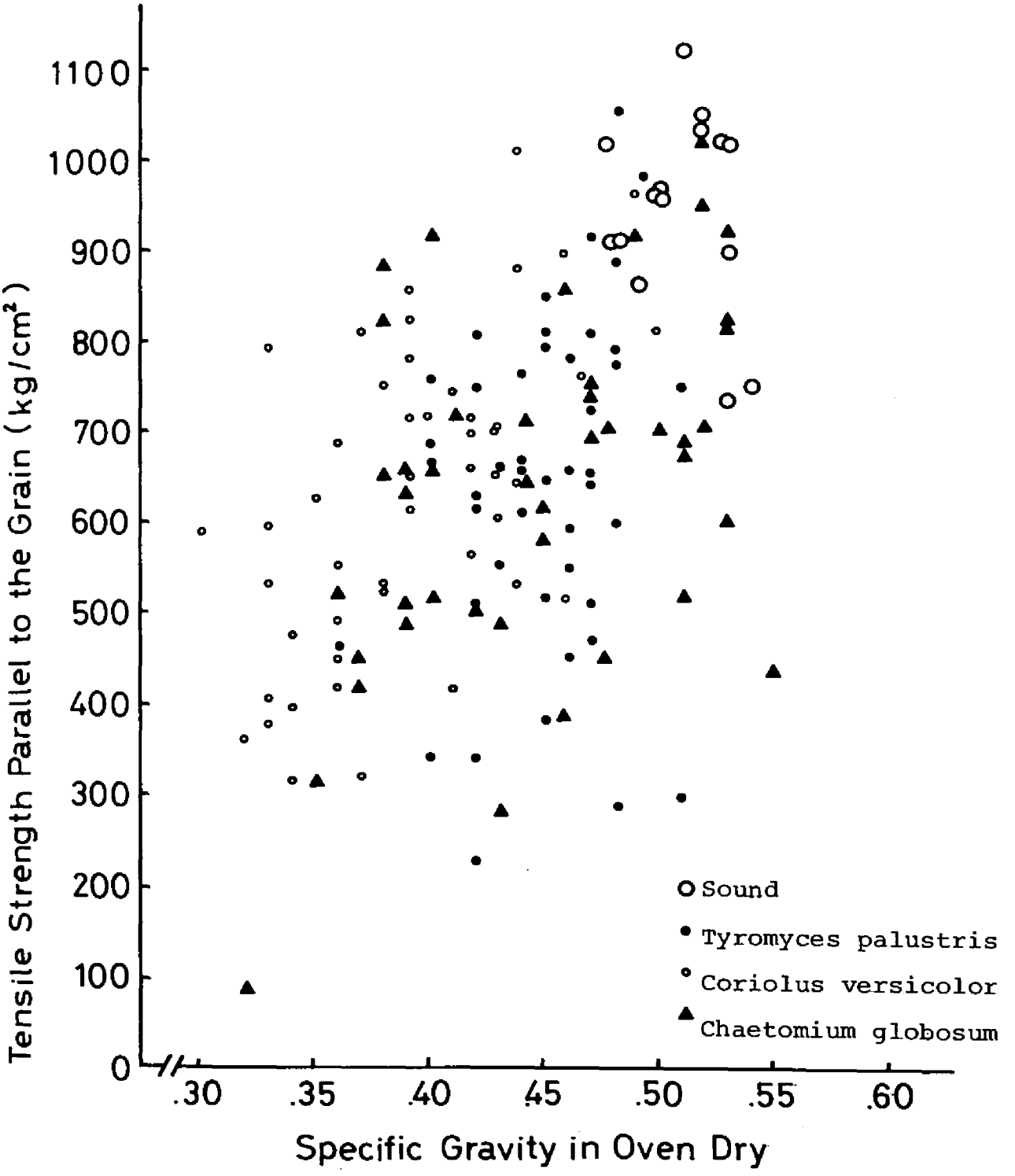



Fig. 17. Reduction in tensile strength (*f*-//) of wood of *Fagus crenata* exposed to the test fungi.

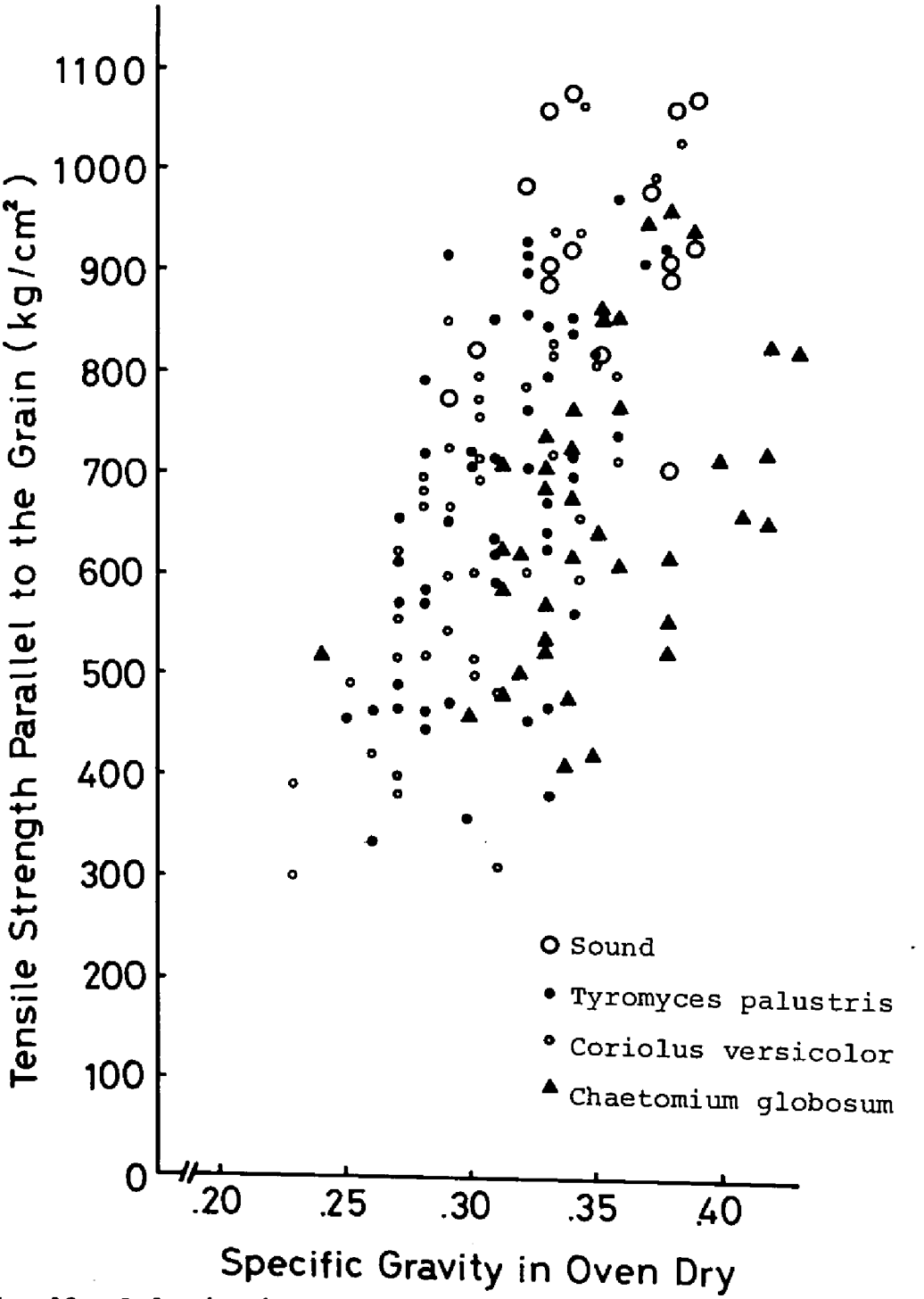



Fig. 18. Reduction in tensile strength (*f*-//) of wood of *Cryptomeia japonica* exposed to the test fungi.

tral portion unsealed. In addition, the specific gravity in the central portion of block was calculated to compare the rate of reduction in each determined strength on the same basis.

Results obtained are summarized as follows:

(1) With the exception of tensile strength, the reduction in strength was not apparent in *C. japonica* exposed to *C. globosum*,

(2) The order of the reduction in strength caused by decay was tensile parallel to the grain > bending > compression perpendicular to the grain > compression parallel to the grain,

(3) The order of the ability to reduce the strengths of wood of *F. crenata* was *T. palustris* (brown rot)> *C. globosum* (soft rot) > *C. versicolor* (white rot).

In agreement with earlier findings[24,26], brown rot fungus caused a rapid drop in strength properties than did white rot fungus. The order of the reduction in strength also coincided approximately with the list by Cartwright and Findlay[33]. The reduction in strength for soft-rotted wood has been studied by a few investigators[4,85,141]. Impact bending strength of wood exposed separately to several soft rot fungi was determined and compared with each other.

On the micro-morphological aspect, the following characteristics have been known for three different types of wood decay[61]:

(1) soft rot; (a) the formation of chains of cavities in the $S_2$ layers of tracheids and fibres,

(2) white rot; (a) the progressive thinning of the secondary cell walls from lumen outwards,

(b) the decomposition occurring uniformly in the regions attacked,

(3) brown rot; (a) no thinning of the secondary cell walls until the very late stages of decomposition,

(b) the irregular patchy attack of the tissues.

Cowling[33] concluded from the analysis of decayed wood that the attack by brown rot fungi brought about a rapid decrease in DP in the cellulose, while white rot fungi caused a gradualer drop in DP. During the attack of beech wood by *C. globosum*[81], the initial increase in DP of about 10 percent of the holocellulose took place, and followed by a gradual decrease. This pattern means that this fungus initially removes holocellulose components with lower molecular weight and then gradually attacks the remaining longer chains. This mode of attack seems to differ from that of brown rot fungi, and certainly from that of white rot fungi.

The walls of wood cells are composed of three groups of structural substances which are classed as framework, matrix and encrusting materials by Wardrop[130]. The framework substance is cellulose which occurs in the form of microfibrils. Cellulose is closely associated with the matrix and encrusting substances in the paracrystalline regions of the microfibrils. The microcapillaries surrounding the cell wall framework are also filled with these amorphous substances. Hemicelluloses are incorporated into the cell wall as the matrix substances. The lignin skeleton that remains after the enzymatic removal of carbohydrates from wood provides clear evidence of the microcapillary system in the cell wall.

A high sensitivity of tensile strength to decay is readily expected on the basis of the theory that DP in the cellulose is an indicator of the tensile strength of wood[79].

There does not exist any generally accepted theory on the fracture in wood which is induced by stress and strain. However, the fracture is accepted as progressive damage, which has three stages[78]: (1) initiation of submicroscopic slips, (2) propagation of microscopic deformations and cracks, and (3) occurrence of macroscopic failures. There are many weak spots in the anatomical structure of wood which create favorable conditions for crack initiation. In the case of decayed wood, additional weak spots which are caused by fungal attack may give a facilitating effect in such a crack initiation.

It is reasonably concluded that the rapid depolymerization of cellulose molecules and the irregular patchy attack of the tissues may cause the rapid drop in strength properties of brown-rotted woods, although there is no experimental evidence for these in the literature and in the present experiment. *C. globosum*, a soft rot fungus, has been ranked in the second order of the ability to reduce the strengths of wood of *F. crenata*. However, it is difficult to explain the mode of strength reduction in relation to the cavity formation in the secondary wall and the different pattern of attack on cellulose polymer by this fungus. Remaining of the sound core which is caused by the progressive destruction from the surface inwards is a macroscopic characteristic of soft-rotted wood[85]. This seems to be responsible at some extent for the rank of *C. globosum*. The distribution of cavity may also influence the

reduction in strength. Liese and Ammer[85], who investigated the reduction in impact bending strength of beech wood exposed to soft rot fungi, reported a close correlation between the number of cells attacked and the strength loss. On the contrary, Zycha[141] could not find such a correlation when he made the similar investigation. Cavity formation creates undoubtedly more or less favorable conditions for crack initiation. However, at the present stage, it is impossible to predict statistically the effect of cavity formation on the strength properties of wood, since it has not been found any generally accepted theory on the fracture.

## 2-2 Changes in infrared spectra of wood on decay[116]

### Materials and Methods

*Preparation of the samples*

The sawdust was collected by cutting the decayed wood blocks of *F. crenata* and *C. japonica* with a small hand-saw at the distance of 6 mm from a cross sectional end of blocks. These blocks were tested for reduction in the compressive strength by decay as described in 2-1. The sawdust was ground up in a mortar to fine powders through a 300 mesh sieve, and the powders were used as samples for infrared spectroscopy.

*Spectroscopic measurement*

Measurement of infrared spectra was made according to the KBr-disk method. The procedure is as follows:

Coarse KBr powder was ground in an agate mortar to pass through a

200 mesh sieve and dried for 48 hours at 130°C. Each sample of the ground wood meal was dried separately *in vacuo* for 8-10 hours at 65°C. Three milligrams of the dried sample was then well mixed with 600 mg of the dried and cooled KBr powder in an agate mortar. The mixture was then pressed in a disk-press at 190 $kg/cm^2$ for 5 minutes under a vacuum condition produced by a normal backing pump. The resulting disk was submitted to spectroscopic measurement through the 1800-650 $cm^{-1}$ region under operation of an infrared spectrophotometer, Japan Spectroscopic DS-402G.

## Results and Discussion

Infrared spectra of the wood samples are recorded separately as shown in Figs. 19-28. On each figure, A shows the spectra of wood decayed by *T. palustris* (brown rot), and B by *C. versicolor* (white rot). The spectra of wood of *F. crenata* exposed to *C. globosum* (soft rot) showed little change despite of the considerable weight loss in blocks. Then, newly prepared samples from the wood meal which had been collected by surface shaving were used in the spectroscopic measurement. The spectra of these samples are shown as C in Figs. 19, 21, 23, 25 and 27. *C. globosum* did not cause appreciable weight loss and significant changes in spectra in wood of *C. japonica*.

Changes in spectra observed in this experiment are described separately in five regions of wave number.

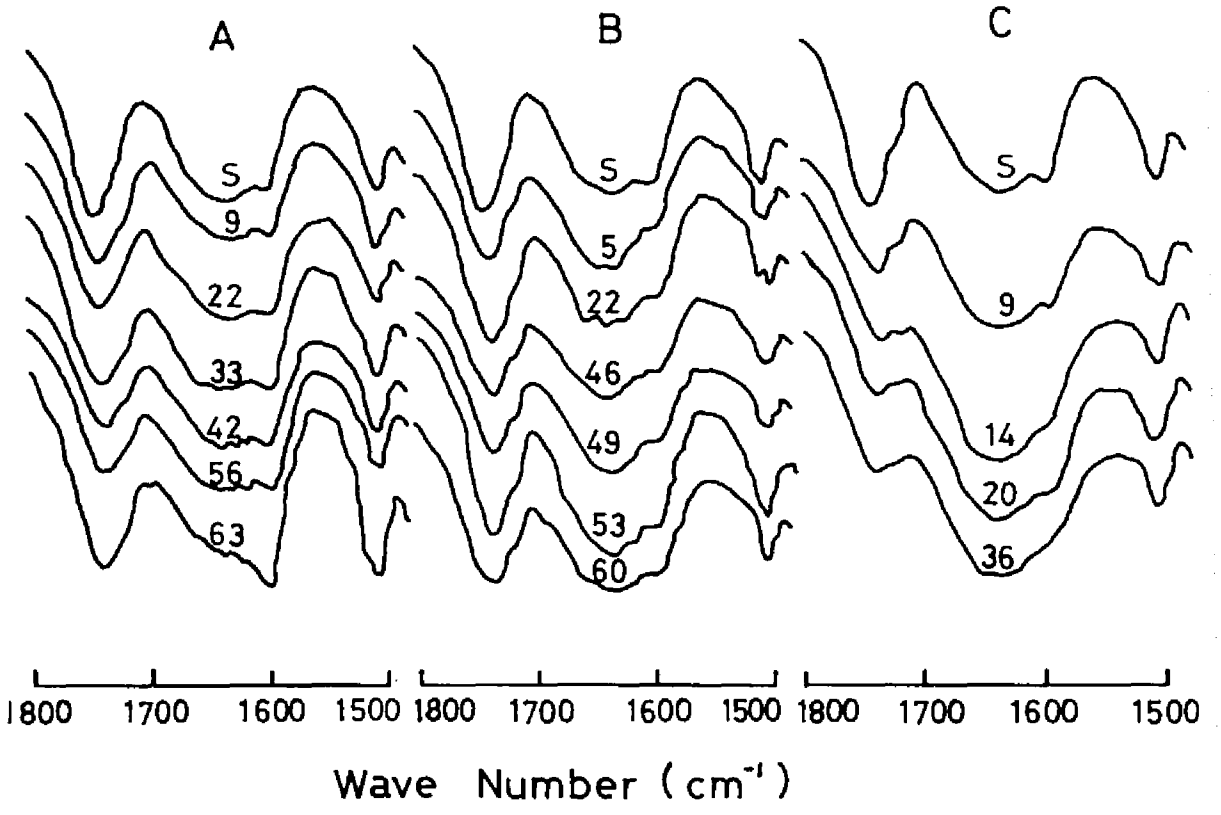



Fig. 19. Infrared spectra of wood of *Fagus crenata* exposed to *Tyromyces palustris* (A), *Coriolus versicolor* (B) and *Chaetomium globosum* (C) in the 1800-1500 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

*In the 1800-1500 $cm^{-1}$ region* (Figs. 19 and 20)

(a) The absorption at 1730 $cm^{-1}$ in *F. crenata* decreased markedly during the degradation by *C. globosum*, whereas only slightly by *T. palustris* and *C. versicolor*.

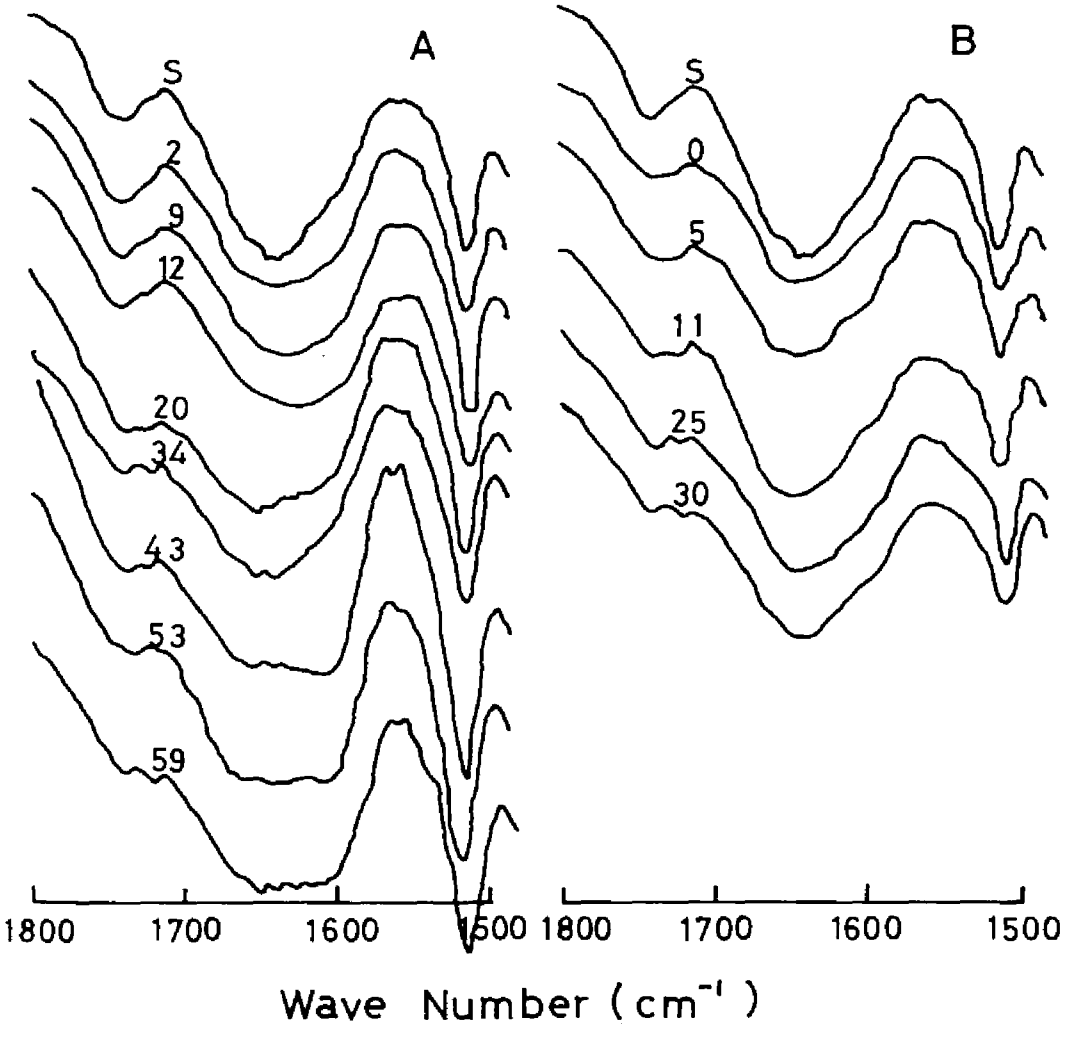



Fig. 20. Infrared spectra of wood of *Cryptomeria japonica* exposed to *Tyromyces palustris* (A) and *Coriolus versicolor* (B) in the 1800-1500 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

(b) The same absorption in *C. japonica* decayed by the two Basidiomycotina fungi decreased constantly. The decrease was observed also in the wood scarcely decayed by *C. globosum*. The occurrence of absorption at about 1715 $cm^{-1}$ was realized in the woods of both species, but strongly

in *C. japonica*.

(c) The absorption band centered at 1640 $cm^{-1}$ was broadened in *C. japonica* decayed by *T. palustris*.

(d) The absorptions at 1595 and 1510 $cm^{-1}$ were sharpened in *F. crenata* decayed by *T. palustris*. The latter absorption in *C. japonica* decayed by the same fungus also increased significantly. These absorptions, however, decreased in *F. crenata* decayed by *C. versicolor* and *C. globosum*. The absorption at 1510 $cm^{-1}$ in *C. japonica* decayed by *C. versicolor* also decreased.

*In the 1500-1300 $cm^{-1}$ region* (Figs. 21 and 22)

(a) The absorption at 1460 $cm^{-1}$ increased with the progress of decay in both woods exposed to *T. palustris*. This was more noticeable for *F. crenata* than for *C. japonica*. Thus, the absorption in *F. crenata* became greater than that at 1420 $cm^{-1}$ as decay proceeds.

(b) The absorption at about 1405 $cm^{-1}$ which was not present in original sound wood was visualized after decay, especially in *F. crenata* decayed by *C. globosum* and in *C. japonica* by *C. versicolor*.

(c) The absorption at 1330-1310 $cm^{-1}$ increased slightly in *F. crenata* exposed to *T. palustris*, while it decreased in *C. japonica* exposed to the same fungus.

*In the 1300-1100 $cm^{-1}$ region* (Figs. 23 and 24)

(a) The broad absorption band at 1300-1200 $cm^{-1}$ in *F. crenata* exhibited the different shape with the progress of decay by *T. palustris*, and that was characterized by the occurrence of two distinct absorptions at 1270 and 1230 $cm^{-1}$. These absorptions were present originally in

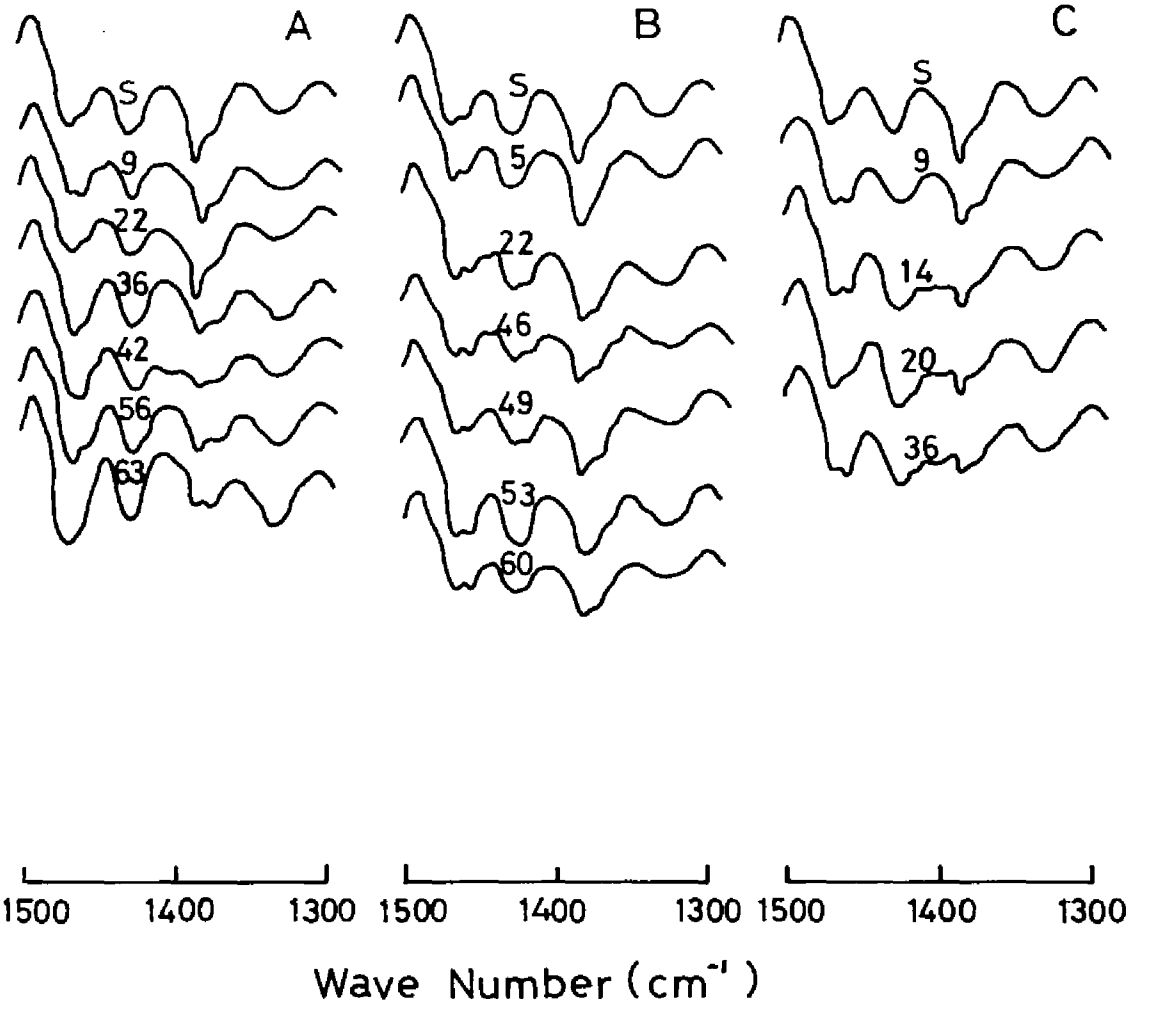



Fig. 21. Infrared spectra of wood of *Fagus crenata* exposed to *Tyromyces palustris* (A), *Coriolus versicolor* (B) and *Chaetomium globosum* (C) in the 1500-1300 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

sound wood of *C. japonica* and increased similarly with the progress of decay by the same fungus.

(b) The broader band at 1200-1000 $cm^{-1}$ weakened progressively, as a whole, as decay proceeds. Details in the change, however, varied with

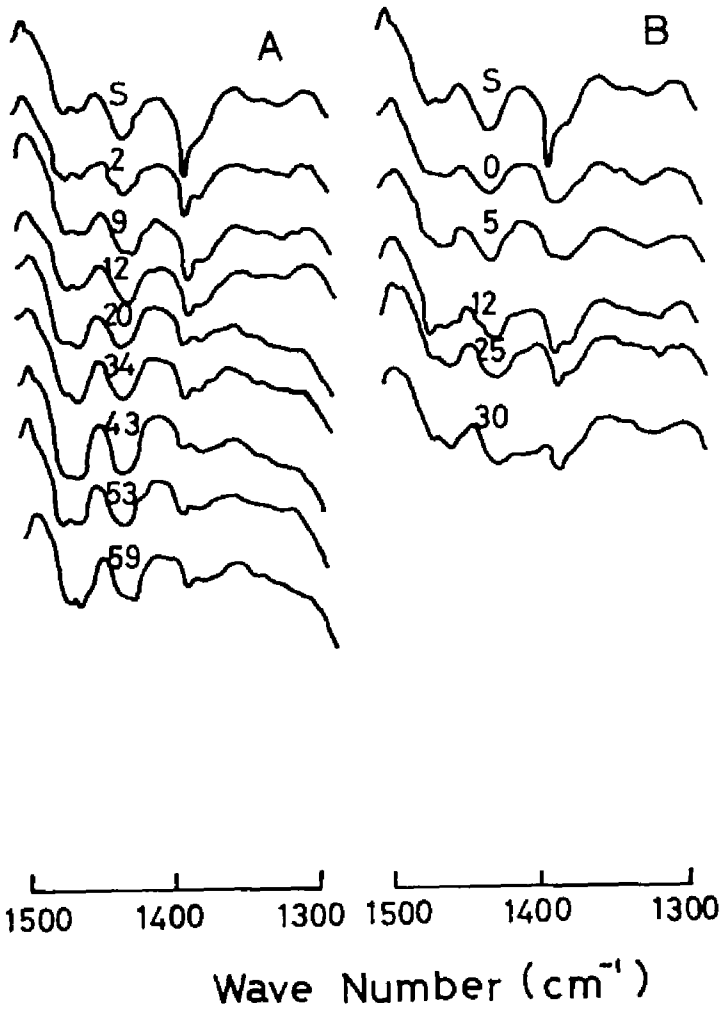



Fig. 22. Infrared spectra of wood of *Cryptomeria japonica* exposed to *Tyromyces palustris* (A) and *Coriolus versicolor* (B) in the 1500-1300 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

the wood and fungal species. The absorption at 1160 $cm^{-1}$ decreased in *F. crenata* exposed to *T. palustris* and *C. globosum*, and in *C. japonica* to *T. palustris*. The absorption at 1120 $cm^{-1}$ increased in *F. crenata* decayed by *T. palustris* and *C. globosum*, but not in *C. japonica* by *T.*

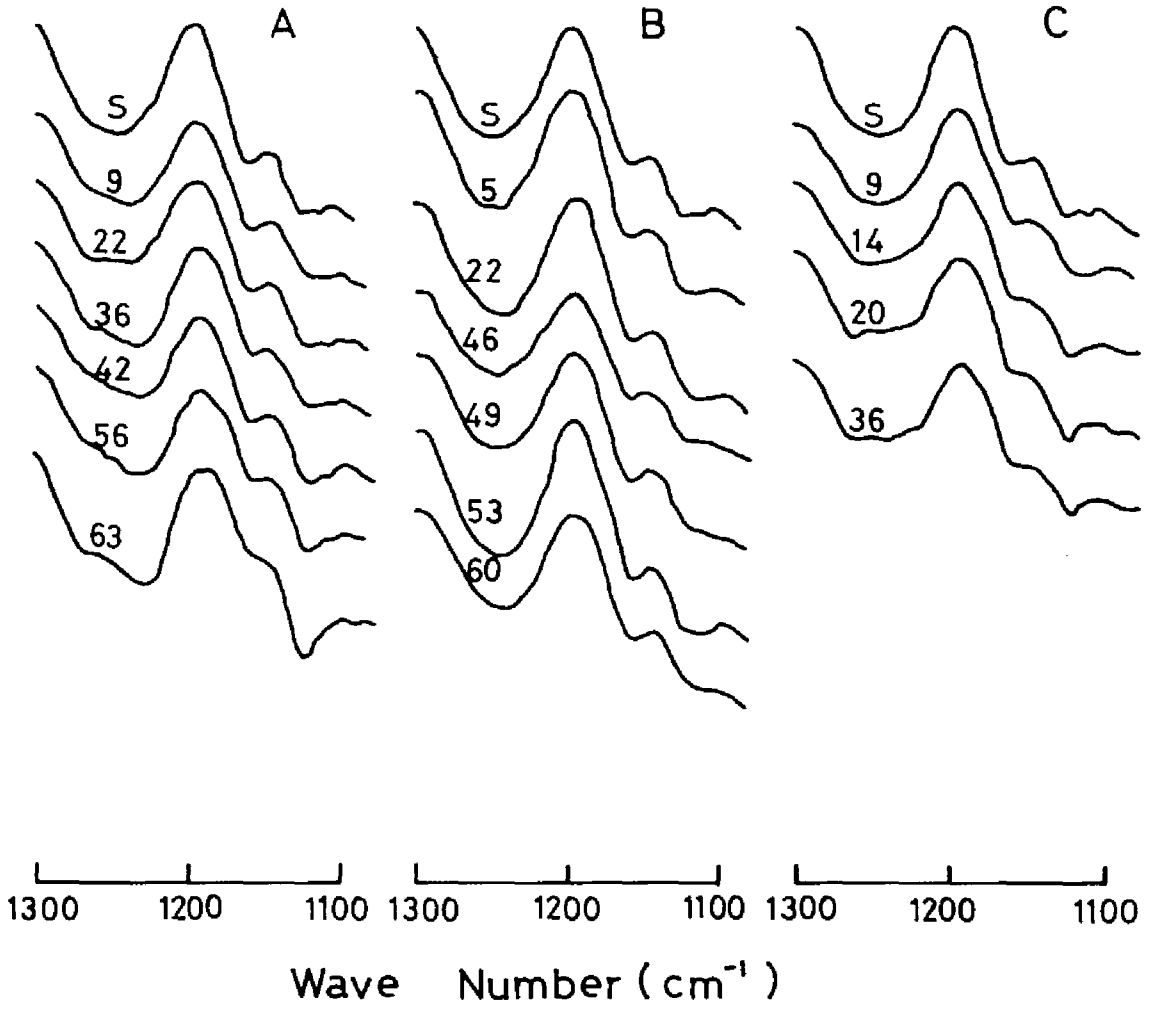



Fig. 23. Infrared spectra of wood of *Fagus crenata* exposed to *Tyromyces palustris* (A), *Coriolus versicolor* (B) and *Chaetomium globosum* (C) in the 1300-1100 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

*palustris* and *C. versicolor*.

*In the 1100-900 $cm^{-1}$ region* (Figs. 25 and 26)

(a) The absorption band at 1050-1030 $cm^{-1}$ in *F. crenata* was divided into two weak absorptions at 1050 and 1030 $cm^{-1}$ after decay by *T. palust-*

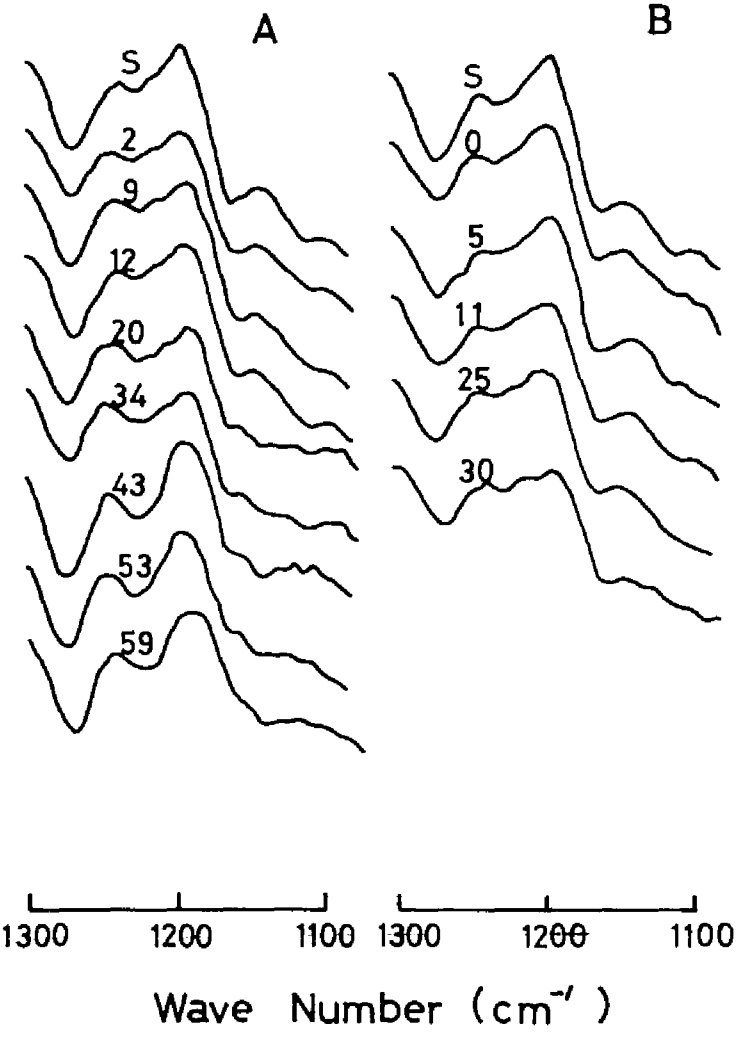



Fig. 24. Infrared spectra of wood of *Cryptomeria japonica* exposed to *Tyromyces palustris* (A) and *Coriolus versicolor* (B) in the 1300-1100 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

*ris.*

(b) These absorptions were present in *C. japonica* before decay. The absorption at 1030 $cm^{-1}$ became greater than that at 1050 $cm^{-1}$ with the progress of decay by *T. palustris* and *C. versicolor*.

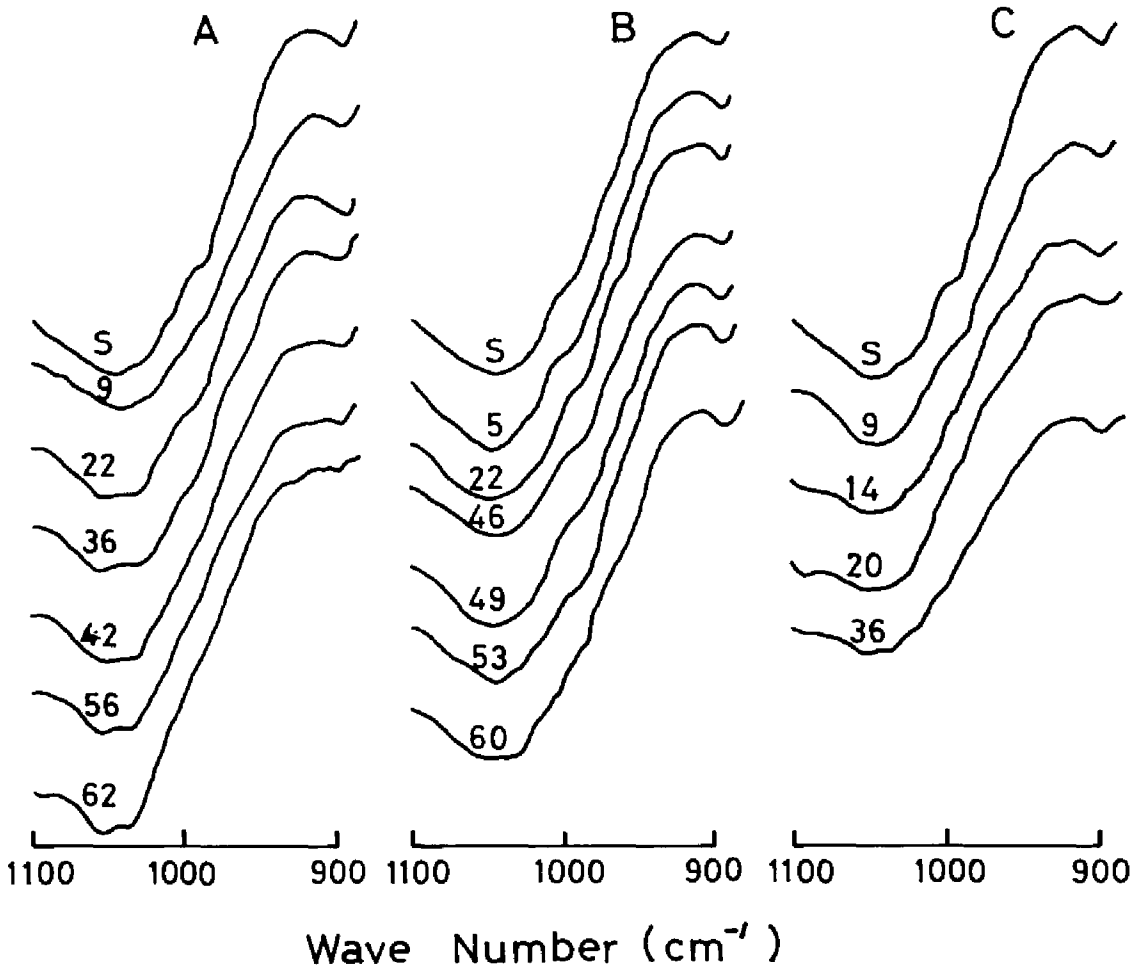



Fig. 25. Infrared spectra of wood of *Fagus crenata* exposed to *Tyromyces palustris* (A), *Coriolus versicolor* (B) and *Chaetomium globosum* (C) in the 1100-900 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

*In the 900-650 $cm^{-1}$ region* (Figs. 27 and 28)

(a) The absorption at 895 $cm^{-1}$ decreased progressively in *F. crenata* decayed by *T. palustris* and *C. globosum*. Such a decrease was also observed in *C. japonica* decayed by *T. palustris*.

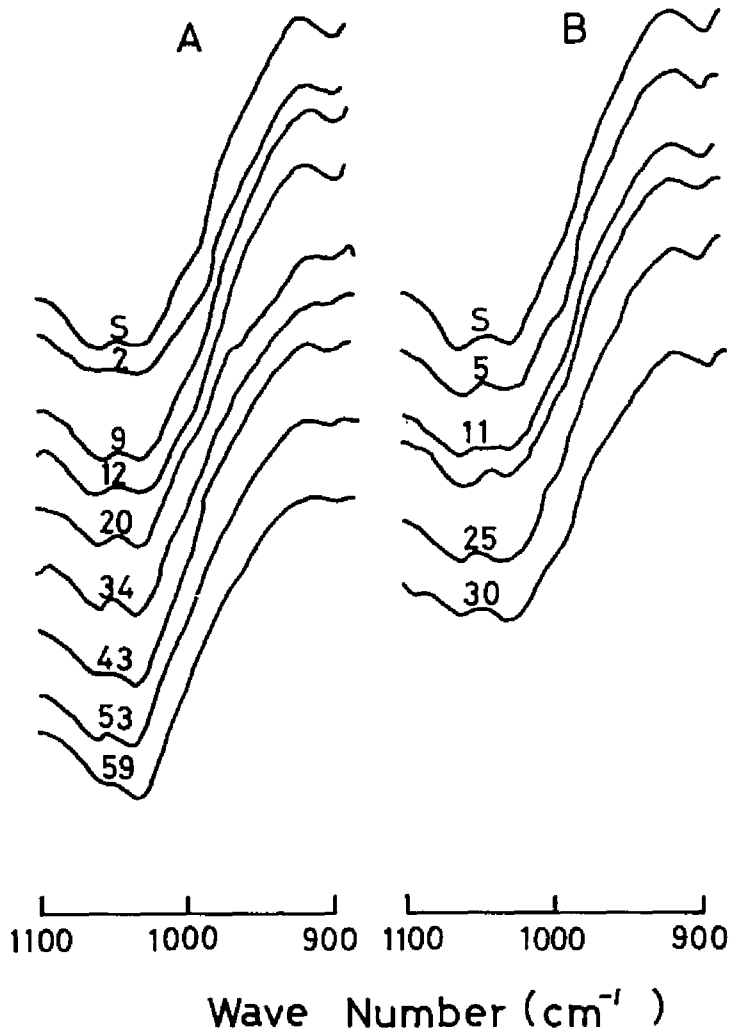



Fig. 26. Infrared spectra of wood of *Cryptomeria japonica* exposed to *Tyromyces palustris* (A) and *Coriolus versicolor* (B) in the 1100-900 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

(b) The decrease in the same absorption was not found in *F. crenata* exposed to *C. versicolor* but slightly in *C. japonica*.

The absorption at 1730 $cm^{-1}$ decreased rapidly in *F. crenata* exposed to *C. globosum*. This was not noticeable in *C. japonica* exposed to the

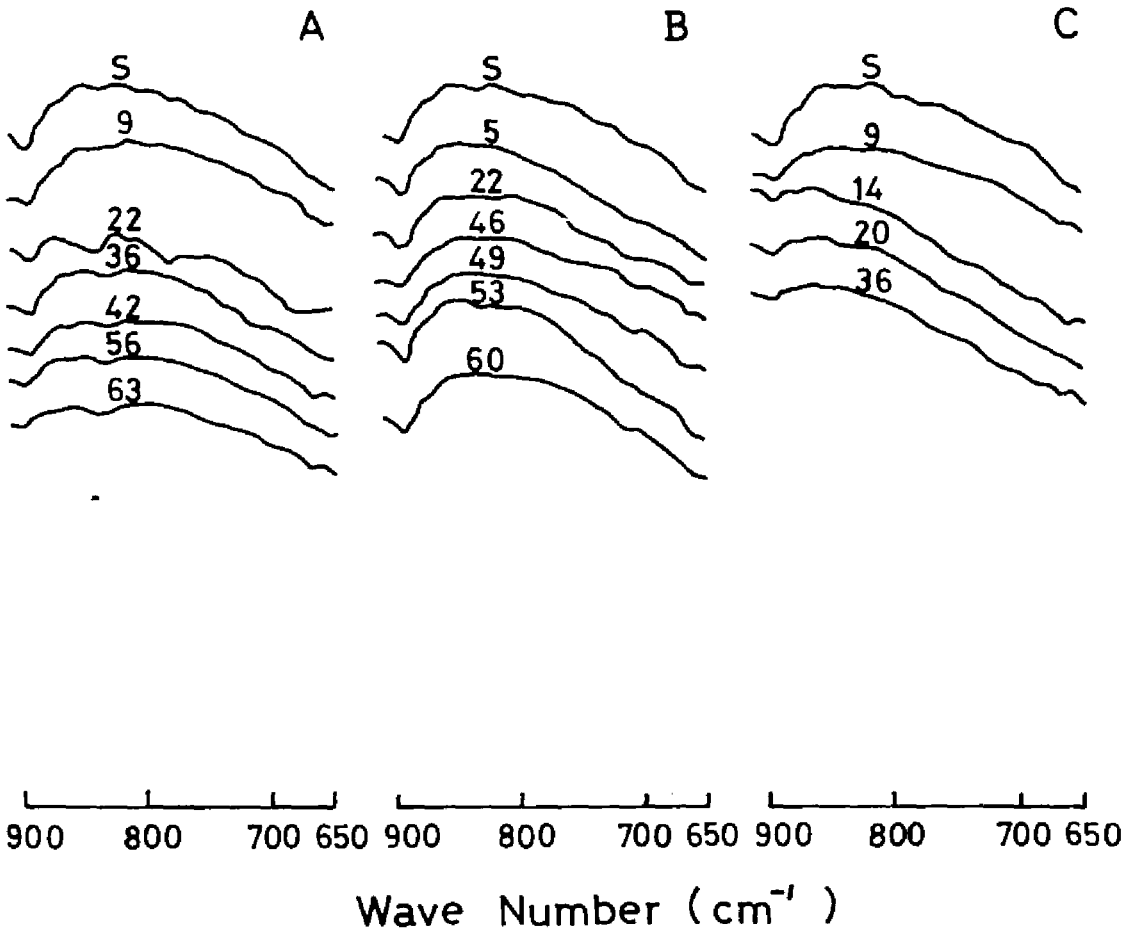



Fig. 27. Infrared spectra of wood of *Fagus crenata* exposed to *Tyromyces palustris* (A), *Coriolus versicolor* (B) and *Chaetomium globosum* (C) in the 900-650 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

same fungus and in *F. crenata* to *T. palustris* and *C. versicolor*. This absorption is assigned to the C=O stretching vibration of carboxyl and acetyl groups in xylan, precisely the *O*-acetyl (4-*O*-methyl glucurono) xylan in hardwoods and the arabino-(4-*O*-methyl glucurono) xylan in soft-

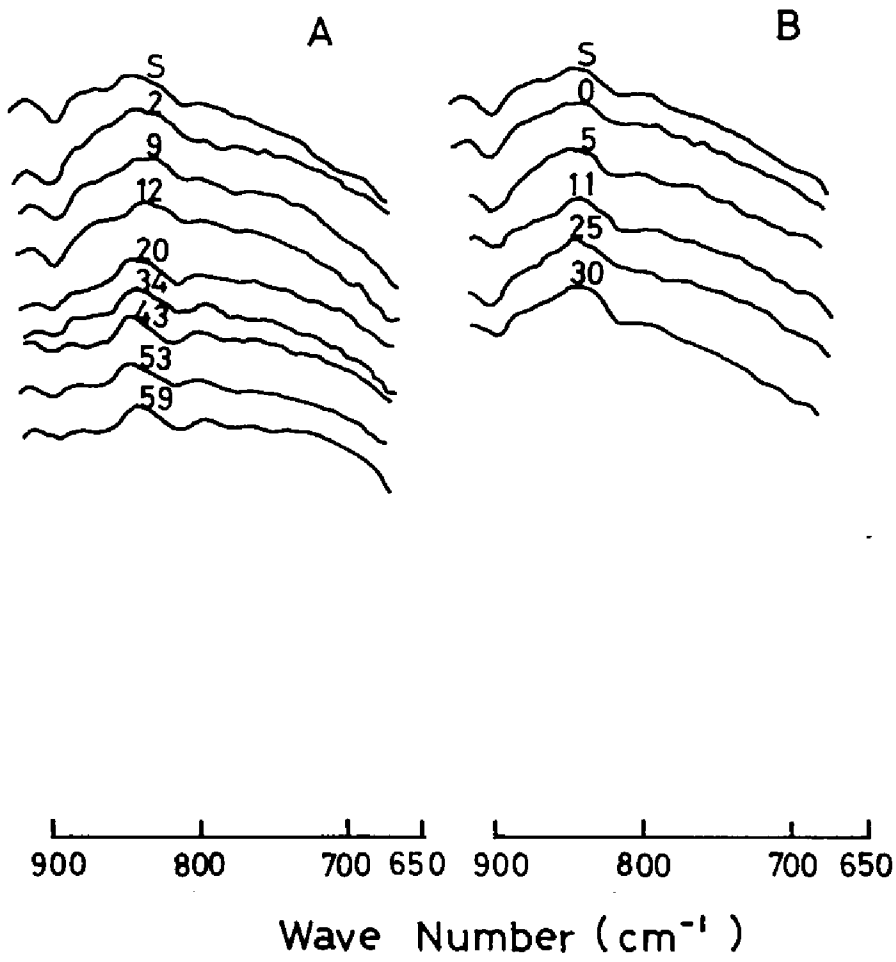



Fig. 28. Infrared spectra of wood of *Cryptomeria japonica* exposed to *Tyromyces palustris* (A) and *Coriolus versicolor* (B) in the 900-650 $cm^{-1}$ region. S: Sound wood, Figure on spectra: % weight loss in wood block.

woods[55]. The absorption at 1460 and 1230 $cm^{-1}$ are due to the $CH_2$ deformation vibration in lignin and xylan with benzene ring vibration in lignin, and to the acetyl and carboxyl vibration in xylan with C=O stretching vibration in lignin, respectively[55]. These absorptions did

not change in *F. crenata* exposed to *C. globosum* and *C. versicolor* but increased in the same wood decayed by *T. palustris*. Among several absorptions refered to cellulose and hemicelluloses, absorptions at 1380, 1160 and 895 $cm^{-1}$ decreased in *F. crenata* exposed to *C. globosum* and *T. palustris*. These results suggested that *C. globosum* was characterized by the specific action on carboxyl and acetyl groups in hemicelluloses and that *C. globosum* and *T. palustris* attacked actively cellulose and hemicelluloses in the decay of hardwoods.

The absorptions at 1595 and 1510 $cm^{-1}$ are due to the well known stretching vibrations of the benzene ring in lignin. These absorptions increased in both woods decayed by *T. palustris* but decreased in those by *C. versicolor* and in *F. crenata* by *C. globosum*. The five absorptions at 1460, 1420, 1270, 1230 and 1030 $cm^{-1}$ are also refered to lignin. Among these, the absorption at 1460 $cm^{-1}$ is assigned to the $CH_2$ deformation with benzene ring vibration and that at 1420 $cm^{-1}$ is to the $CH_3$ bending vibration[55]. The other absorptions are due to the C=O stretching vibrations, but they are also associated with cellulose or hemicelluloses[55]. These five absorptions did not decrease in the brown-rotted woods but showed little change in the white-rotted and soft-rotted ones. Changes in absorptions due to lignin in the soft-rotted wood were thus quite different from those in the brown-rotted one, while but similar to those in the white-rotted one.

*C. globosum* has been shown to cause depletion of the lignin in beech wood[81,105,108]. The lignin remaining in the decayed wood was deficient in methoxyl and acid-soluble more than that in the sound wood[81]. Thus,

this soft rot fungus can bring about at least some degradation of lignin, but it is not known what specific sequential changes are involved[71]. In comparison of the characteristics of soft rot with other types of wood decay[105], carbohydrate depletion with little lignin attack has been emphasized as the similarity to brown rot, and the gradual increase in alkali solubility as that to white rot. However, the results obtained here suggest that soft rot fungi are more active against hardwood lignin than are brown rot fungi.

Infrared spectra of wood exposed to *C. versicolor* were not very different from those of sound wood, with the exception of few absorptions at 1595, 1510 and 1380 $cm^{-1}$. Earlier investigations[22,33] revealed that the solubility of wood in water, 1 % NaOH, and in organic solvents did not change considerably during decay by white rot fungi. This indicates that the rates of production and utilization of degradation products are approximately equal. In accord with this, the polysaccharides[33] and lignin[69] remaining in white-rotted wood at various stages of decay are not very different in properties from these substances in sound wood. Therefore, only small parts of the polymers are attacked at any given time, and the affected portions are completely degraded and assimilated before other parts of the polymers are significantly affected.

The absorptions at about 1715 and 1405 $cm^{-1}$ which were visualized after decay were not discussed here, since their assignments have not been known so far.

The loss of weight in decayed wood indicates the removal of wood substances by decay fungi, in the form of water-soluble compounds, water

and carbon dioxide as the final degradation products in fungal metabolism. In the very early stage in which the substantial loss of weight in wood is still undetected, any of the limited changes in polymer structure must occur. Infrared spectral method was expected to be sensitive to detect such structural changes occurring in the early stage of wood decay. However, as shown in the results, any changes in infrared spectra did not precede the percent of weight loss. The absorptions at 1730, 1380 and 1050 $cm^{-1}$ were more sensitive to decay than other absorptions. Thus, infrared spectral method using KBr-disk preparation proved less effective on the structural analysis of wood constituents at the early stage of decay, but was very useful, as well as the conventional chemical analysis, for the qualitative examination of wood decay.

## 2-3 Utilization of carbohydrates[117)]

### Materials and Methods

*Carbon source and medium*

The following carbohydrates were added as the sole carbon source to the basal medium:

L-arabinose, D-glucose, D-mannose, D-galactose, D-glucuronic acid, D-galacturonic acid, D-maltose, CMC (carboxymethyl cellulose sodium salt, 0.5 to 0.7 in degree of substitution, and 300 to 450 in degree of polymerization), and crude xylan extracted from wood of *Fagus crenata*.

Abrams's solution[1)] was used as the basal nutrient medium. The composition is as follows:

$NH_4NO_3$ 3.0 g, $KH_2PO_4$ 2.5 g, $K_2HPO_4$ 2.0 g, $MgSO_4 \cdot 7H_2O$ 2.0 g and distilled water 1000 ml.

Amounts of carbon source were 10 g per litre for xylan and 20 g for other carbohydrates. The carbon sources were sterilized in vapor of propylene oxide and added to the autoclaved Abrams's solution. When using D-glucuronic acid and D-galacturonic acid as carbon source, pH values of these uronic acid media were adjusted to 6.5 with sterilized sodium hydroxide. The experiments were mainly carried out in 500 ml shaking flasks containing 50 ml of nutrient solution. Three to five replicates per each kind of carbon source were tested.

Prior to experiments, the mycelia of the test fungus were transfered to Petri dishes containing malt agar substrate. Sterilized cellophane disks of 8 mm diameter were laid on the agar plate. After covering the agar plate and cellophane disks by mycelial development, the disks with mycelia were removed and rinsed in sterilized distilled water. The flasks were inoculated with these mycelial disks and incubated at 28°C in a shaker for 21 days.

*Determination of growth rate and consumption rate of carbon sources*

Growth rate was determined every three days as mycelial dry weight per flask by drying for 20 hours at 90°C after filtering the nutrient medium. In some experiments with solid agar media containing equal amount of xylose or glucuronic acid as in the nutrient solution, growth of the mycelium was measured in millimeters per day. In addition, the pH of each culture filtrates was measured every three days.

The culture filtrates were analysed for the amount of remaining

carbohydrates by methods of Bertland[93], and Tollens and Krober[18]. Samples taken from the xylan-containing solutions were hydrolysed with 1 *N* sulfuric acid for 6 hours at 100°C, and followed by neutralization with barium hydroxide and passage through Amberlite IR-120 and IR-4B. The effluents and washings containing neutral sugars were concentrated and analysed for pentose content by method of Tollens and Krober[18].

*Test fungi*

A test fungus was *Chaetomium globosum* Kunze (IAM 8059). *Coriolus versicolor* Quél. (FES 1030) and *Tyromyces palustris* Murr. (FES 0507) were occasionally used for comparison.

*Preparation of crude xylan from beech wood*

Beech wood (*Fagus crenata* Blume) sawdust, 40 to 60 mesh, was extracted with ethanol-benzene (1:1). The extractive-free wood (350 g) was suspended in water (3.5 l) and treated with glacial acetic acid (20 ml) and sodium chlorite (80 g) by occasional stirring for 1 hour at 70-80°C. Then, the same amounts of additional reagents were added without cooling. The heating was continued at the same temperature range for an additional hour. This treatment was repeated again in the same condition.

The chlorite holocellulose was extracted with 5 % sodium hydroxide under nitrogen gas for one overnight at a room temperature. The mixture was filtered with suction into a flask containing an excess of glacial acetic acid, and 95 % ethanol was added to the filtrate and washings. The resulting precipitate was collected by centrifuging, and then washed repeatedly with 5 % ethanol, ethanol and ether, and dried on potassium

hydroxide *in vacuo*. A crude xylan was obtained as light grayish powder.

The contents of lignin and uronic acid of the sample was determined as Klason lignin and uronic anhydride according to TAPPI Standards T 13m-54 and Browning's method[19], respectively.

## Results and Discussion

Growth of *C. globosum* on various carbon sources other than crude xylan and consumption rate of some carbon sources are shown in Figs. 29 and 30. Xylose, mannose, glucose, galactose and maltose were available to *C. globosum*. Of these sources, however, galactose apparently gave slower fungal growth, although the final mycelial yield was nearly equal to other sources. Cochrane[29] reviewed the carbon nutrition of fungi and concluded that galactose was utilized by most fungi but was not usually so good source as glucose. Results obtained here suggest that *C. globosum* is adapted to metabolize galactose during incubation, and that the metabolic activity to galactose may not be constitutive for the fungus.

There are many reports showing that glucose is a good carbon source for many fungi including wood-decaying fungi and that xylose and mannose are equivalent to glucose[29,99]. In the present investigation, however, glucose was slightly inferior to xylose and mannose in daily growth rate. In the metabolism of glucose by fungi, three different pathways have been established, namely EMP (Embden-Meyerhof-Parnas) pathway, HMP (hexose monophosphate) pathway and ED (Enter-Douroroff) pathway. Mannose and galactose are converted into fructose-6-phosphate via glucose and/or

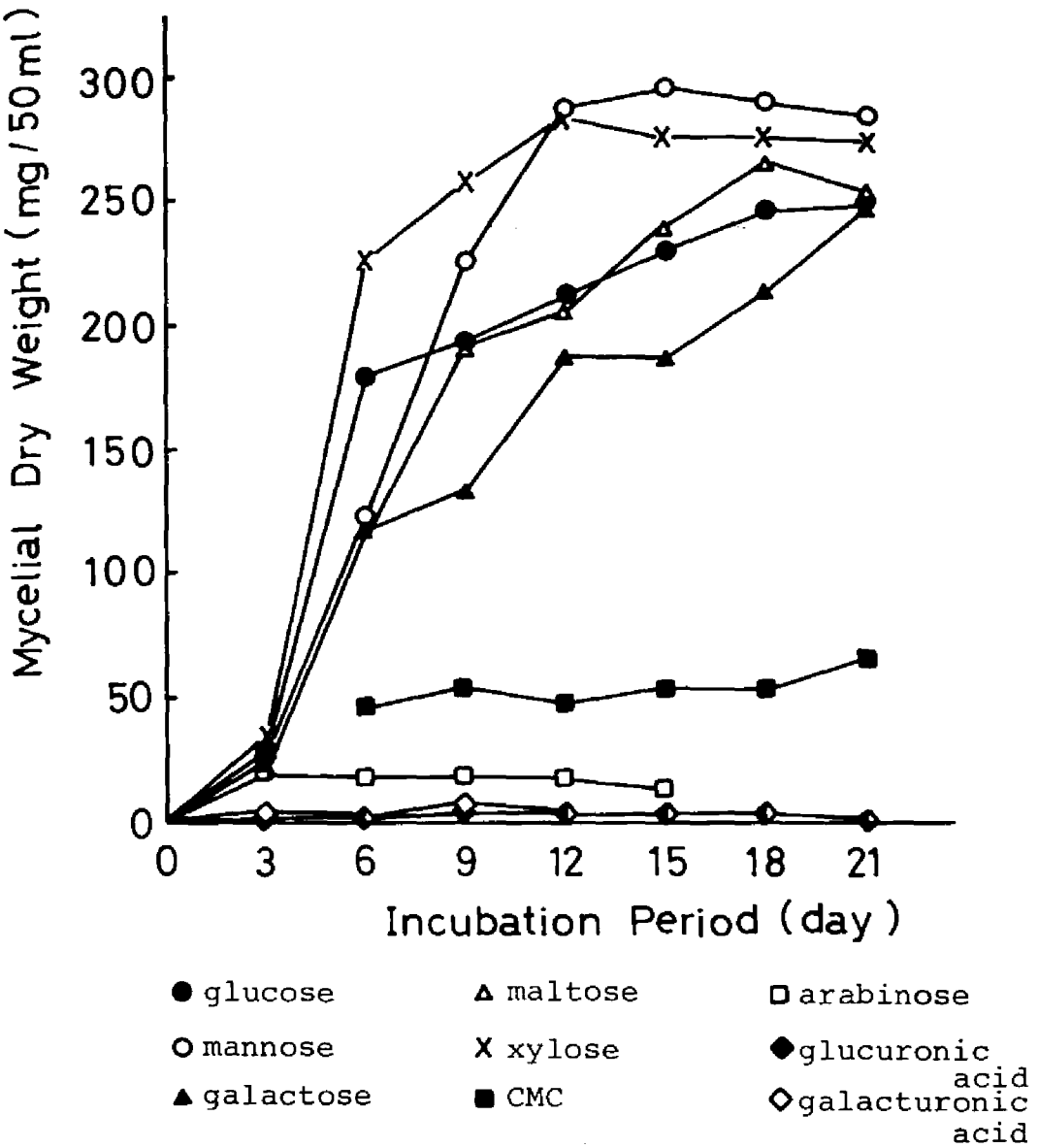



Fig. 29. Mycelial dry weight of *Chaetomium globosum* grown in Abrams's medium with various carbon sources.

phosphorylated derivatives of these sugars, and derived into the EMP and HMP pathways[29], whereas xylose is firstly converted into xylulose-5-phosphate via xylulose and connected to HMP pathway to produce fructose-6-phosphate[36]. Rapid growth and consumption in xylose medium seem to reflect active isomerization, phosphorylation and other reactions,

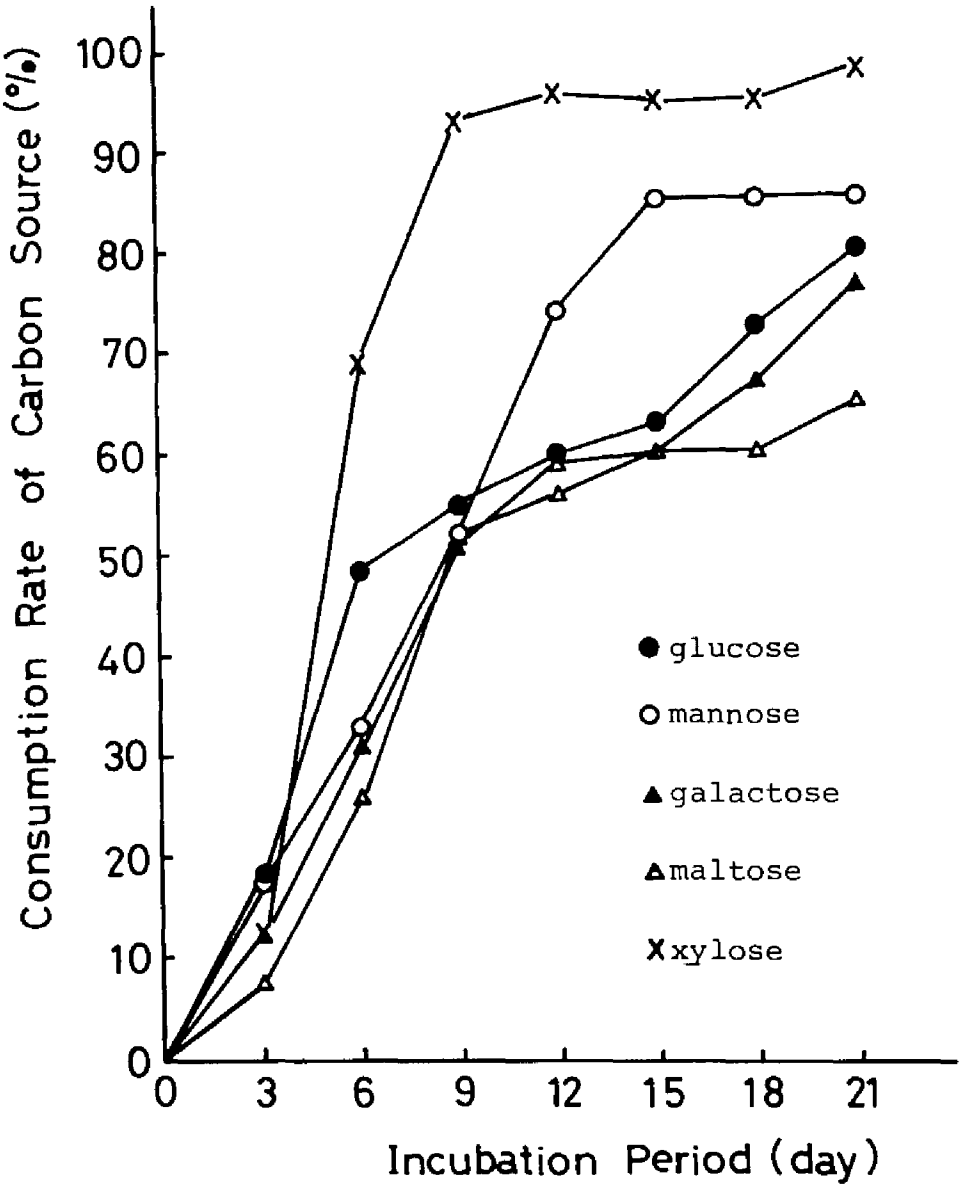



Fig. 30. Consumption rate of five carbon sources by *Chaetomium globosum* grown in Abrams's medium.

leading the major participation of HMP pathway in *C. globosum*.

Xylose and mannose are the main constituents of wood hemicelluloses. Percent of amount of xylose in extractive-free basis is ranging 12 to

26 in hardwoods and 5 to 10 in softwoods[126]. From the present results it can be seen that mannose is a good carbon source with xylose for *C. globosum*. Keilich et al.[67], in agreement with this result, reported that *C. globosum* was able to degrade both glucomannans and glucurono-xylan. As to the enzyme activity, however, they found that xylanase activity of this fungus was higher than mannanase activity.

Changes of economic coefficient in five carbon sources are shown in Fig. 31. Economic coefficient is one of the methods to determine the efficiency of conversion of carbon source to mycelium. It is defined by the formula;

$$\frac{\text{mycelium dry weight (g)}}{\text{amount of carbon compound consumed (g)}} \times 100$$

Economic coefficient is maximal when respiratory carbon dioxide and soluble metabolic products are minimal in quantity. Very low values reflect probably the production of significant amounts of soluble compounds in high-carbohydrate media[99].

As mentioned above, xylose and mannose were utililized more rapidly than other sources, reflecting rapid growth on these sources. However, economic coefficient of xylose maintained the same moderate level throughout the incubation period, whereas that of mannose increased rapidly in early period and then decreased gradually. This suggests that transition from accumlation of metabolic product to mycelial formation is abrupt in the utilization of mannose by *C. globosum*.

Although consumption rate of maltose was slower than that of other sources, economic coefficient of the source was throughly kept at higher

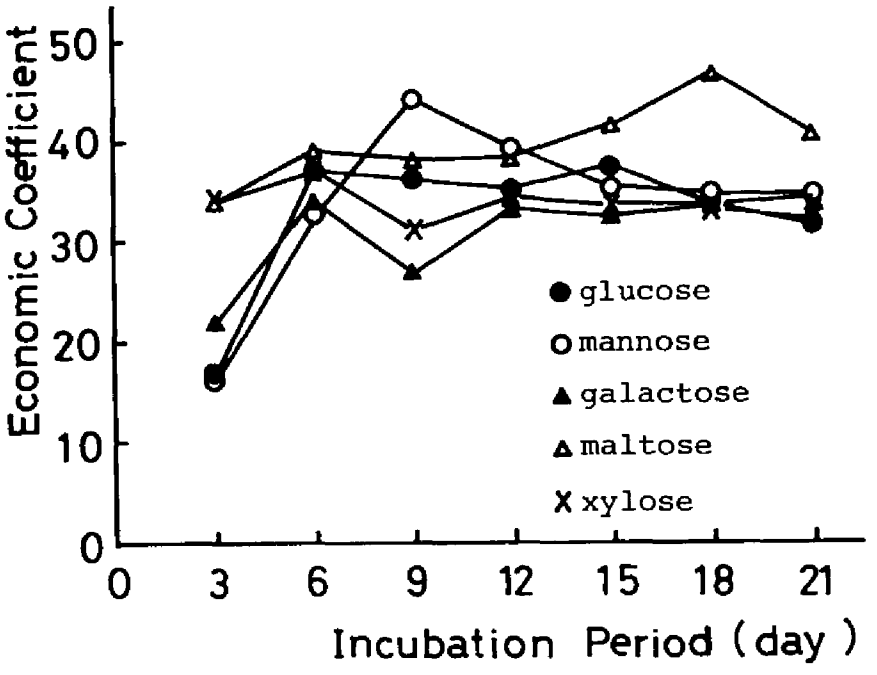



Fig. 31. Efficiency of conversion of five carbon sources to mycelium of *Chaetomium globosum*.

level, and mycelial yield was equivalent to other sources. This indicates the presence of maltase and the effective use of maltose for mycelial formation.

L-Arabinose occurs as a hemicellulose component, though the content is very low. In contrast to D-xylose, this sugar was not utilized at all by *C. globosum*. The fact probably indicates the lack of metabolic pathway — arabinose → ribulose → ribulose 5-phosphate → xylulose 5-phosphate[36]. Inability to utilize both L and D isomers of arabinose has been reported on many fungi[58].

Glucuronic acid and galacturonic acid which are occurring in wood

components of glucuronoxylan and galactoglucomannan were not utilized at all, even when the pH values in these uronic acid media were adjusted to 6.5 with sodium hydroxide solution (Fig. 32). On the solid agar medium which contained glucuronic acid as the sole carbon source and was adjusted to pH 6.5 with sodium hydroxide solution, poor growth of *C. globosum* was observed better than on no carbon medium, but it was apparently lesser than of *C. versicolor* and *T. palustris* (Fig. 33). This indicates that the ability of *C. globosum* to utilize the uronic acid in wood hemicellulose is very low.

Growth on CMC medium was also very poor. Growth on the third day was not measured due to the difficulty of separating the mycelium from the carbon source. As a cellulolytic activity of *C. globosum* was not estimated enzymatically, the reason for such poor growth was uncertain. Carboxymethyl group in the substrate may be supressive to the growth of the fungus, although this fungus is undoubtedly the truly cellulolytic fungus.

Growth, consumption rate and economic coefficent of three fungal species in xylan-containing medium are shown in Figs. 34-36. The contents of lignin and uronic anhydride of this source were 6.25 % and 5,56 %, respectively. As mentioned in "Materials and Methods", determination of pentosan was carried out with the neutral fraction from hydrolyzed culture filtrates. Correction to the fulfural yield from uronic acids was not made. Maximum mycelial yields of test fungi were almost the same as shown in Fig. 34. However, *C. globosum* grew more rapidly and kept the same level of growth for longer period than did *C. versi-*

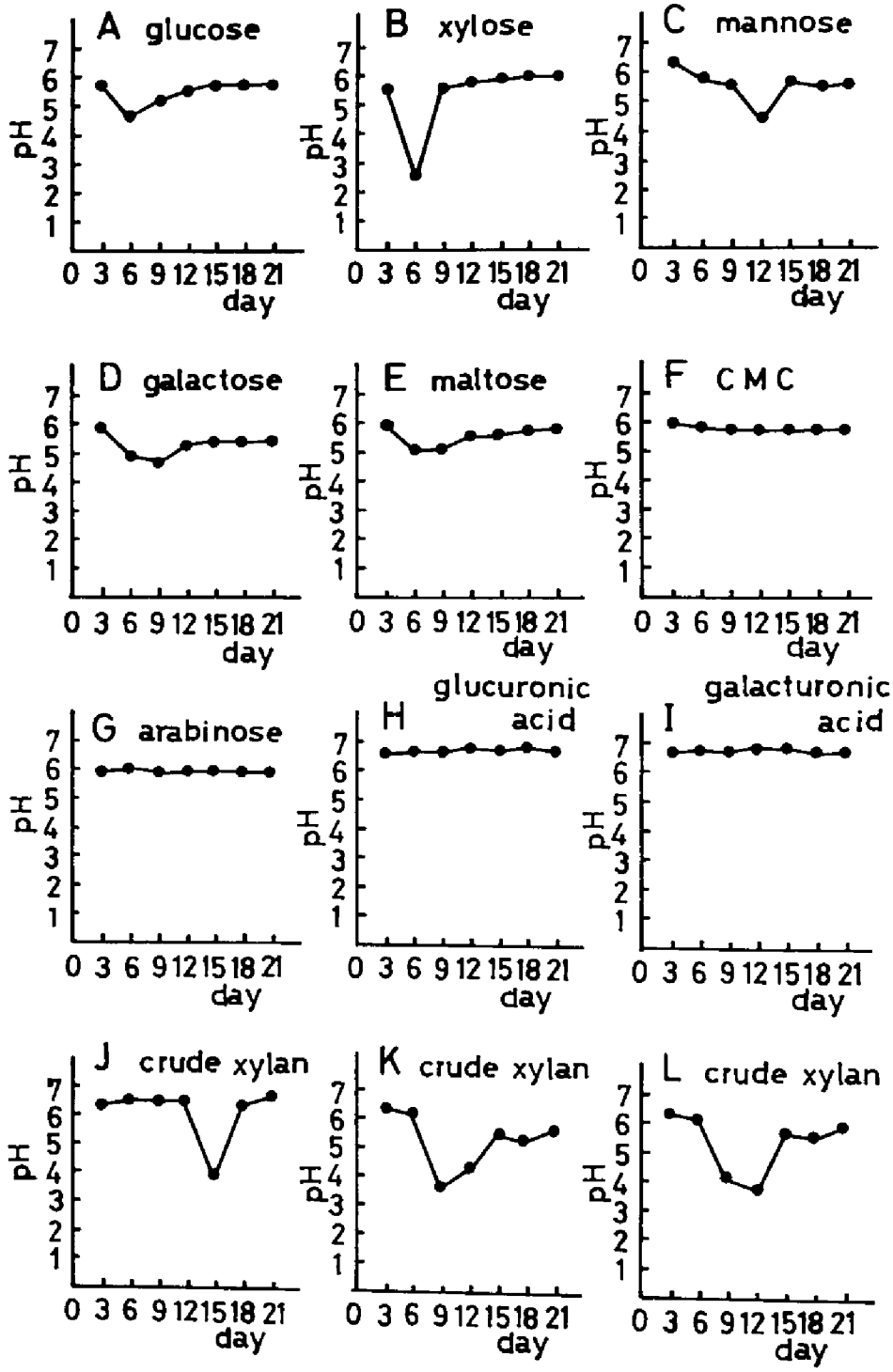



Fig. 32. Change in pH value of Abrams's medium with various carbon sources during incubation (A-J: *Chaetomium globosum*, K: *Coriolus versicolor*, L: *Tyromyces palustris*).

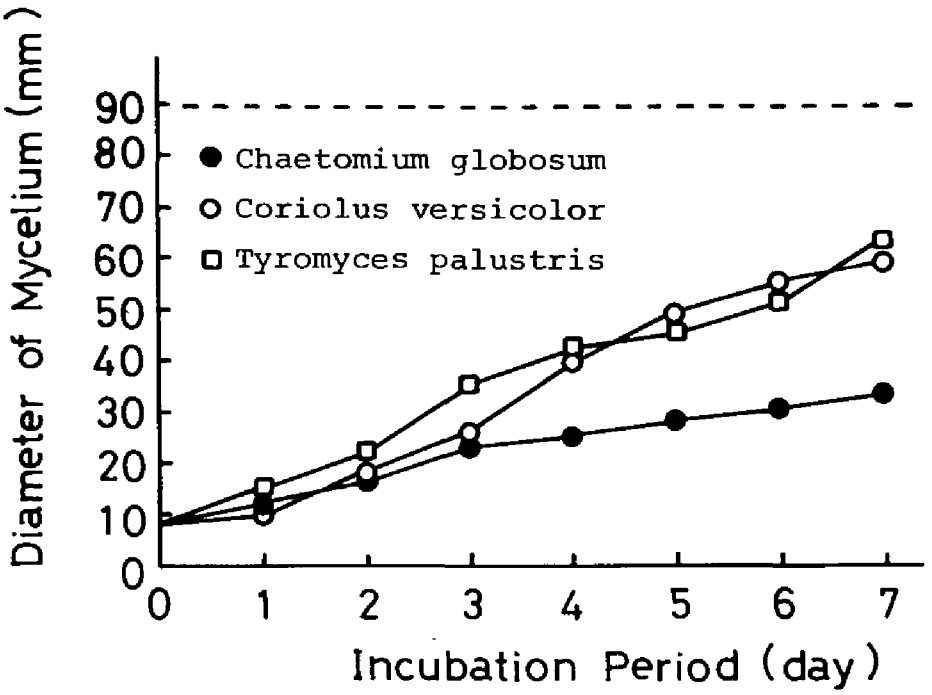



Fig. 33. Radial growth of three fungal species grown on Abrams's agar medium with glucuronic acid as sole carbon source (Broken line shows the diameter of Petri dish).

*color* and *T. palustris*. Economic coefficient for *C. globosum* kept a higher level throughout the incubation period, although the progressive increase of consumption rate showed fairly similar pattern to those of other fungi. Radial growths of these fungi on solid agar medium containing xylose as the sole carbon source were almost the same until the fifth day, but after that day *C. globosum* was superior to other fungi (Fig. 37).

As shown in Fig. 32, the pH value of each nutrient medium from which carbon source was available to test fungi exhibited a similar pattern of transition, that is, a considerably rapid fall and recovery of the value during the course of incubation. Such a pattern has been usually observed for many fungi and seems to reflect the accumlation of some acidic inter-

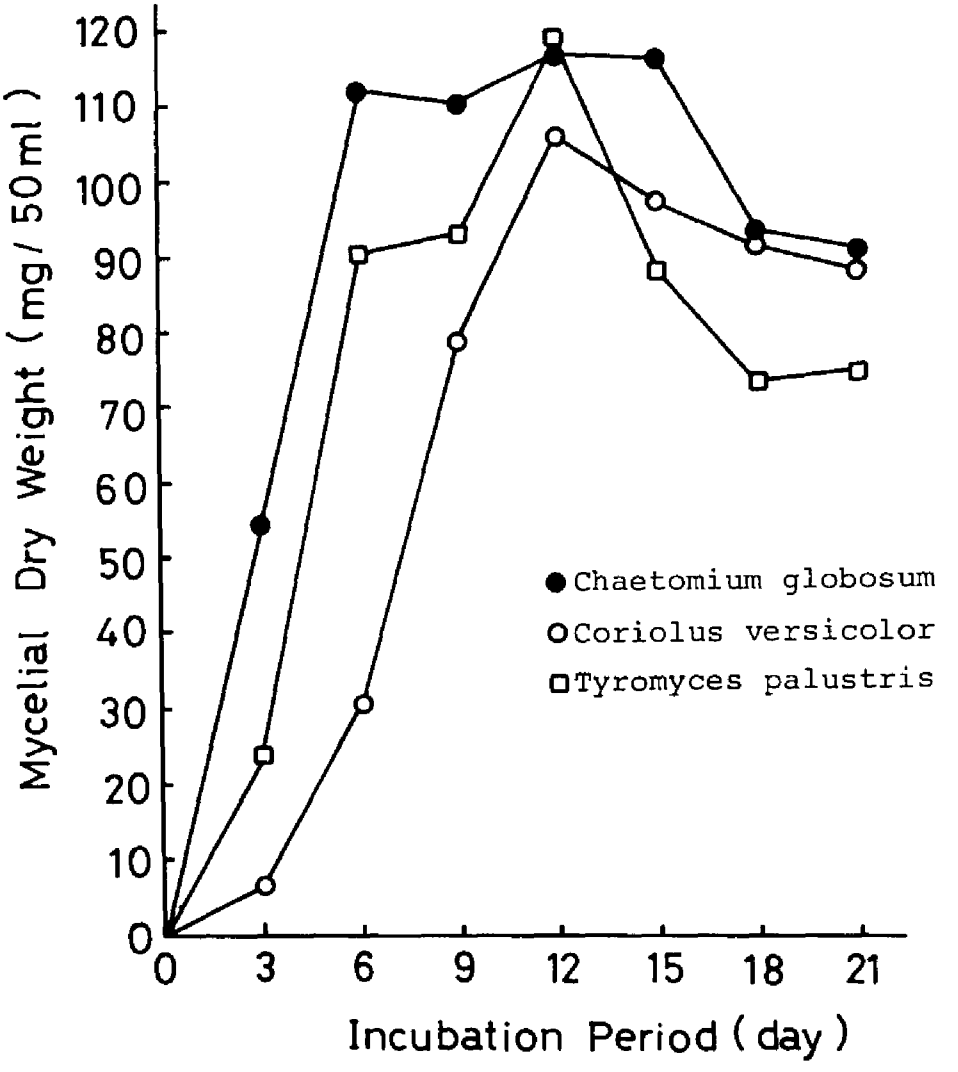



Fig. 34. Mycelial dry weight of three fungal species grown in Abrams's medium with crude xylan as sole carbon source.

mediate products.

From the present results that *C. globosum* is able to utilize xylose and xylan, it can be seen that this soft rot fungus has an active pathway to metabolize these mono- and polysaccharides, indicating the possi-

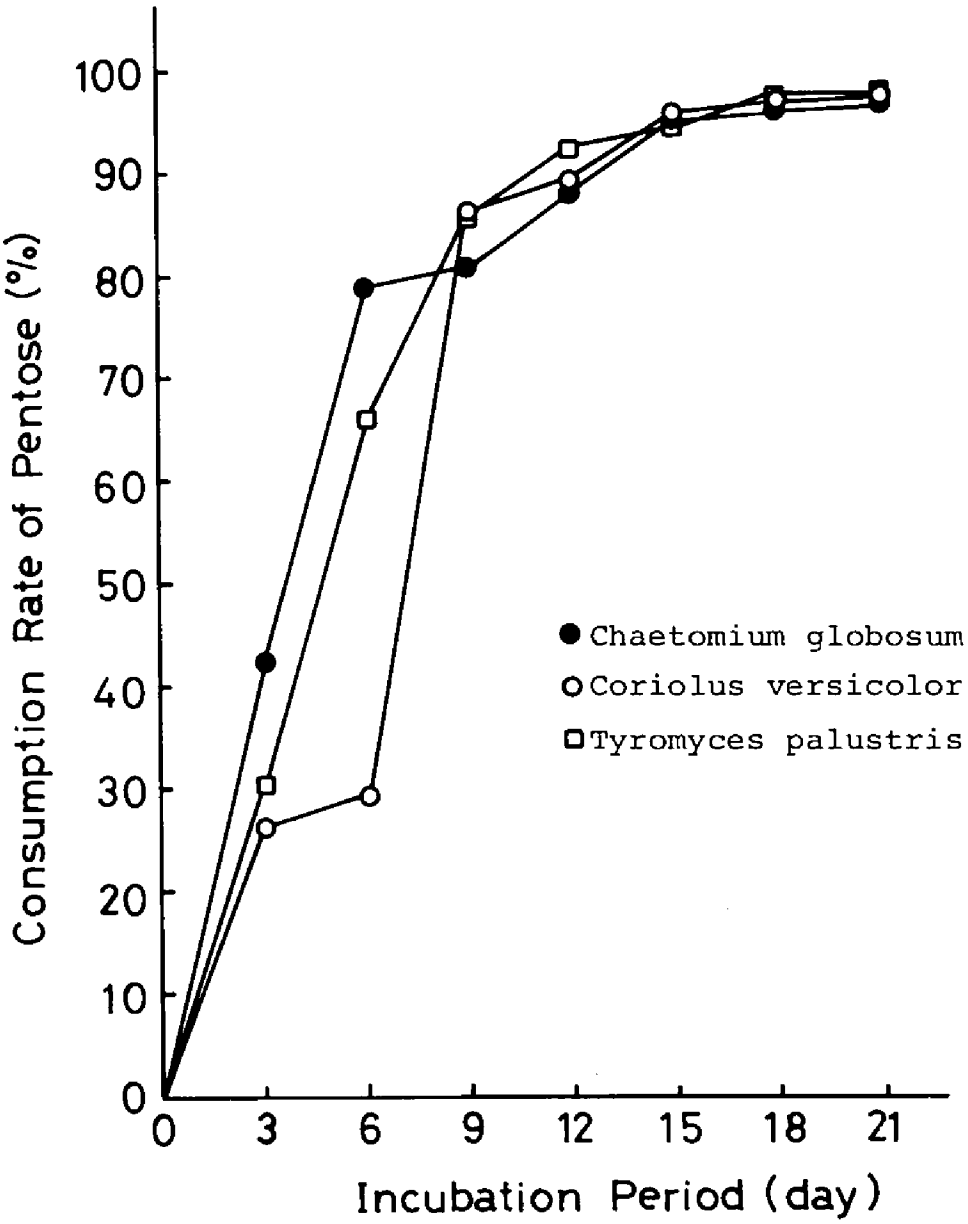



Fig. 35. Consumption rate of pentose by three fungal species grown in Abrams's medium with crude xylan as sole carbon source.

ble connection to the greater susceptibility of hardwoods to soft rot fungi.

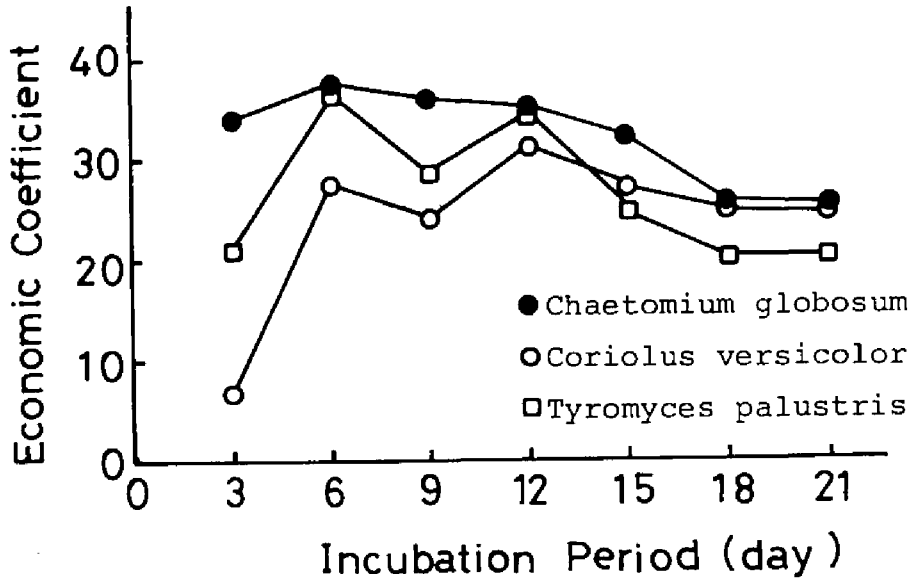



Fig. 36. Efficiency of conversion of crude xylan to mycelium of three fungal species.

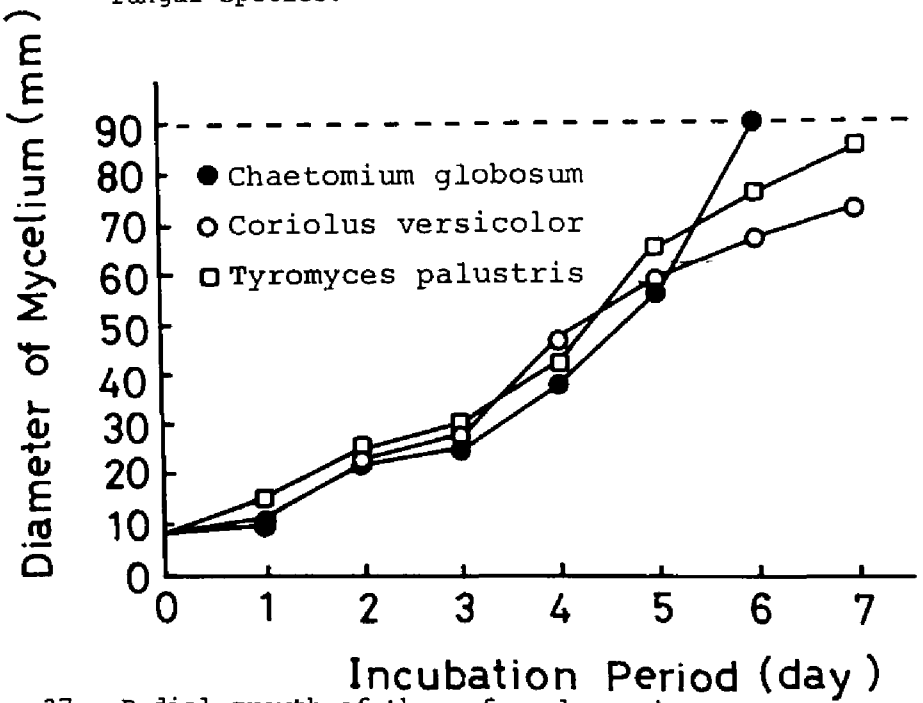



Fig. 37. Radial growth of three fungal species grown on Abrams's agar medium with xylose as sole carbon source (Broken line shows the diameter of Petri dish).

## 2-4 Summary

To achieve a better understanding of the mechanisms of wood degradation by soft rot fungi, change in strength properties and infrared spectra of wood caused by *Chaetomium globosum* were compared with those caused by a white rot fungus, *Coriolus versicolor* and a brown rot fungus, *Tyromyces palustris*. Utilization of carbohydrates by *C. globosum* was also investigated for the same purpose. Results obtained were summarized as follows:

(1) The reduction in strength was clearly demonstrated for *Fagus crenata* (hardwood) exposed to *C. globosum*, but not for *Cryptomeria japonica* (softwood) exposed to the fungus with the exception of tensile strength.

(2) The order of the reduction in strength caused by decay was tensile parallel to the grain > bending > compression perpendicular to the grain > compression parallel to the grain.

(3) The order of the ability to reduce the strengths of wood of *F. crenata* was *T. palustris* > *C. globosum* > *C. versicolor*.

(4) The absorption at 1730 $cm^{-1}$, assigned to the C=O stretching vibration of carboxyl and acetyl groups in xylan, decreased rapidly in both woods of *F. crenata* and *C. japonica* exposed to *C. globosum*, although the substantial loss of weight was not detected in the latter.

(5) The absorption at 1595 and 1510 $cm^{-1}$, assigned to the stretching vibrations of the benzene ring in lignin, increased in both woods exposed to the brown rot, while decreased in both to the white rot and

in *F. crenata* to the soft rot.

(6) D-Xylose, D-mannose, D-glucose, D-galactose, D-maltose and xylan were available to *C. globosum*, whereas L-arabinose, D-glucuronic acid and D-galacturonic acid were not utilized at all.

(7) Rapid rates of growth and consumption of carbon source were demonstrated for xylose- and xylan media inoculated with *C. globosum*.

From the spectral analysis of decayed wood, it is obvious that *C. globosum* is more active against hardwood lignin than is a brown rot fungus used, although preferential depletion of carbohydrates with little lignin attack has been emphasized as the similarity of soft rot to brown rot. Rapid decrease of the absorption at 1730 $cm^{-1}$ in *F. crenata* and rapid utilization of xylose and hardwood xylan by *C. globosum* suggest the possible connection to the greater susceptibility of hardwoods to soft rot fungi. The order of the ability for *C. globosum* to reduce the strengths of wood seems to reflect these degradation pattern of wood constituents and the mode of attack on lignified cell wall generally characterized by cavity formation in secondary wall.

# CHAPTER 3 NATURAL DECAY RESISTANCE OF WOOD AGAINST CHAETOMIUM GLOBOSUM

An apparent wood preference has been recognized for many of the white rot- and brown rot fungi, although there seems to be no absolute specificity involved in. White rot fungi are associated most frequently with decay of hardwoods and brown rot fungi with decay of softwoods. Soft rot fungi seem to attack hardwoods more readily than softwoods. However, these relationships are not generalized due to the lack of data. Soft rot fungi have been isolated frequently even from softwoods serviced in various environments[37,104]. Greaves and Levy[54] investigated on the durability of wood which was subjected to long-term exposure (3300 to 5 years) in widely differing environments. The most intensive destruction, according to them, was found to occur in mining timber in which bacteria and soft rot fungi had combined to erode completely the secondary cell wall of fibres and tracheids. Thus, the higher resistance of softwoods against soft rot fungi which is evidently exhibited in the laboratory tests does not mean necessarily the predominant occurrence of soft rot on hardwoods, and might be overcome at some extent with some fungal/bacterial associations, or any of unknown biological combinations and non-biological effects.

In this chapter, investigations are reported on the natural decay resistance of various wood species against *C. globosum*. It is frequently pointed out that the higher decay resistance of a certain heartwood against Basidiomycotina fungi is often caused by the presence of toxic

extractives in wood[3,101]. However, role of extractives in the higher resistance of wood against soft rot fungi has not yet been studied at all. Thus, heartwood blocks, newly prepared from all species of softwoods and tropical hardwoods used, and from those species of temperate hardwoods which were designated resistant against *C. globosum*, were extracted with hot methanol and exposed to this fungus.

## 3-1 Temperate hardwoods[118]

### Materials and Methods

*Wood species and preparation of test block*

The wood species used in the experiment are listed in Table 7. Most of important species in Japan are involved in 138 species of 89 genera over 41 families. Considering the well established trend of increasing decay resistance from the innermost to the outermost heartwood[54,87], heartwood samples were taken as near as possible from the intermediate portion. Sampling of sapwood samples, if possible, was made also from the middle part. The size of test block was decided as 2.0 ($t$) × 2.0 ($r$) × 0.5 ($l$) (cm), considering the surface action of soft rot fungi to the wood block.

*Test fungi*

*Chaetomium globosum* Kunze (IAM 8059) was used as a test fungus.

*Decay test*

The decay test was done by the sand-block method using cylindrical glass bottles described in Chapter 1. Three blocks each in two bottles

were used for each wood species. The composition of the nutrient solution was the same as that described in Chapter 2. Test blocks were soaked in sterilized distilled water after fumigation with propylene oxide, since soft rot fungi have a preference for wet conditions. The temperature was maintained at 28°C during the 8 week-incubation period. The decayed blocks were cleaned of mycelium, and then dried to constant weight in an oven at 105°C. The percent of weight loss was calculated from initial and final weights.

*Extraction of test blocks with hot methanol*

Extractives are those substances which are removed from wood by extraction with neutral solvents. Although the same sequence of extractions is not applicable to all woods because of the variable composition of the extractives, methanol is preferentially used as the first solvent for successive extraction. Heartwood blocks from resistant species based on decay tests were extracted with hot methanol for 8 hours and exposed to the test fungus. The content of methanol extractives was calculated by reweighing the extracted block.

## Results and Discussion

A few schemes on the classification of timbers on the basis of decay resistance have been proposed over the past years[28,46,100]. In Japan, however, classification scheme has not been established yet. In this experiment, on the basis of the weight loss in beech wood (*Fagus crenata*) and frequency distribution of weight loss, the resistance of hardwood species was divided into five classes as follows:

Table 7. Hardwood timber species used in the decay test.

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H- 1 | Salicaceae | *Populus maximowiczii* Henry | Doronoki | Japan poplar | W |
| H- 2 | " | *Populus nigra* L. var *italica* Koehne | Seiyo-hakoyanagi | Lombardy poplar | K |
| H- 3 | " | *Salix sachalinensis* Fr. Schm. | Onoeyanagi | (Willow) | A |
| H- 3 | | | | | |
| H- 4 | " | *Salix alopechroa* Kimura | Saigoku-kitsune yanagi | (Willow) | K |
| H- 5 | " | *Toisusu urbaniana* Kimura var. *schneideri* Kimura | Tokachi-yanagi | (Willow) | W |
| H- 5 | | | | | |
| H- 6 | Juglandaceae | *Juglans mandchurica* Maxim. subsp. *sieboldiana* Kitam. | Onigurumi | Japanese walnut | W |
| H- 7 | " | *Pterocarya rhoifolia* Sieb. et Zucc. | Sawagurumi | Japanese wingnut | W |
| H- 7 | | | | | |
| H- 8 | Betulaceae | *Ostrya japonica* Sarg. | Asada | Japanese hophornbeam | W |
| H- 9 | " | *Carpinus japonica* Blume | Kumashide | (Hornbeam) | W |
| H- 9 | | | | | |
| H- 10 | " | *Carpinus tschnoskii* Maxim. | Inushide | (Hornbeam) | W |
| H- 11 | " | *Corylus sieboldiana* Blume | Tsunohashibami | (Hazel) | A |
| H- 12 | " | *Betula ermani* Cham. | Dakekanba | Dakekaba | W |
| H- 12 | | | | | |
| H- 13 | " | *Betula grossa* Sieb. et Zucc. | Mizume | (Birch) | W |
| H- 13 | | | | | |
| H- 14 | " | *Betula platyphylla* Sukat. var. *japonica* Hara | Shirakanba | Shirakaba | W |
| H- 14 | | | | | |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H- 15 | Betulaceae | *Alnus firma* Sieb. et Zucc. var. *hirtella* Franch. et Sav. | Miyama-yashabushi | (Alder) | K |
| H- 16 | " | *Alnus hirsuta* Turcz. | Keyamahannoki | (Alder) | W |
| H- 17 | " | *Alnus hirsuta* Turcz. var. *sibirica* C. K. Schneid. | Yamahannoki | (Alder) | K |
| H- 18 | " | *Alnus japonica* Steud. | Hannoki | Japanese alder | K |
| H- 19 | Fagaceae | *Fagus crenata* Blume | Buna | Japanese beech | W |
| H- 19 | | | | | |
| H- 20 | " | *Quercus acutissima* Carr. | Kunugi | Kunugi oak | W |
| H- 21 | " | *Quercus mongolica* Fisch. | Mizunara | Karafuto oak | W |
| H- 22 | " | *Quercus serrata* Thunb. | Konara | Konara oak | A |
| H- 22 | | | | | |
| H- 23 | " | *Quercus variabilis* Blume | Abemaki | (Oak) | K |
| H- 24 | " | *Quercus acuta* Thunb. | Akagashi | (Oak) | W |
| H- 25 | " | *Quercus gilva* Blume | Ichiigashi | (Oak) | W |
| H- 25 | | | | | |
| H- 26 | " | *Quercus glauca* Thunb. | Arakashi | Ring-cupped oak | K |
| H- 27 | " | *Quercus hondai* Makino | Hanagagashi | (Oak) | W |
| H- 27 | | | | | |
| H- 28 | " | *Lithocarpus amygdafolia* Sieb. et Zucc. | Amigashi | (Lithocarpus) | W |
| H- 29 | " | *Castanea crenata* Sieb. et Zucc. | Kuri | Japanese chestnut | W |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H- 30 | Fagaceae | *Castanopsis cuspidata* Schottky | Tsuburajii | (Western chinquapin) | W |
| H- 31 | " | *Castanopsis* sp. | (Shii) | (Castanopsis) | W |
| H- 31 | | | | | |
| H- 32 | " | *Passania edulis* Makino | Matebashii | (Passania) | K |
| H- 33 | Ulmaceae | *Ulmus davidiana* Planch. var. *japonica* Nakai | Harunire | Nire-elm | W |
| H- 33 | | | | | |
| H- 34 | " | *Ulmus lacinata* Mayr | Ohyo | Ohyo-nire | W |
| H- 35 | " | *Zelkova serrata* Makino | Keyaki | Keyaki | W |
| H- 35 | | | | | |
| H- 36 | " | *Celtis sinensis* Pers. var. *japonica* Nakai | Enoki | Japanese hackberry | W |
| H- 37 | " | *Aphananthe aspera* Planch. | Mukunoki | (Aphananthe) | W |
| H- 38 | Moraceae | *Morus alba* L. | Kuwa | Silkworm mulberry | W |
| H- 38 | | | | | |
| H- 39 | " | *Morus bombycis* Koidz. | Yamaguwa | (Mulberry) | A |
| H- 39 | | | | | |
| H- 40 | " | *Broussonetia kazinoki* Sieb. | Kouzo | Paper mulberry | A |
| H- 41 | " | *Cudrania tricuspidata* Bureau | Hariguwa | (Cudrania) | K |
| H- 42 | Trocho- | *Trochodendron aralioides* | Yamaguruma | (Trochodendron) | W |
| H- 42 | dendraceae | Sieb. et Zucc. | | | |
| H- 43 | Cercidi- | *Cercidiphyllum japonicum* | Katsura | Katsura | W |
| H- 43 | phyllaceae | Sieb. et Zucc. | | | |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H- 44 | Magnoliaceae | *Magnolia liliflora* Desr. | Mokuren | (Cucumber tree) | K |
| H- 45 | " | *Magnolia kobus* DC. | Kobushi | Thunber's Magnolia | K |
| H- 46 | " | *Magnolia praecocissima* Koidz. | Ezokobushi | (Cucumber tree) | W |
| H- 47 | " | *Magnolia obovata* Thunb. | Honoki | Japanese cucumber tree | W |
| H- 48 | " | *Magnolia salicifolia* Max. | Tamushiba | (Cucumber tree) | A |
| H- 49 | Lauraceae | *Cinnamomum camphora* Sieb. | Kusunoki | Camphorwood | W |
| H- 49 | | | | | |
| H- 50 | " | *Cinnamomum japonicum* Sieb. | Yabunikkei | (Camphorwood) | W |
| H- 50 | | | | | |
| H- 51 | " | *Machilus thunbergii* Sieb. et Zucc. | Tabunoki | (Machilus) | W |
| H- 52 | " | *Lindera erythrocarpa* Makino | Kanakuginoki | (Lindera) | W |
| H- 52 | | | | | |
| H- 53 | " | *Neolitsea aciculata* Koidz. | Inugashi | (Neolitsea) | W |
| H- 53 | | | | | |
| H- 54 | " | *Actinodaphne lancifolia* Meissn. | Kagonoki | (Actinodaphne) | W |
| H- 54 | | | | | |
| H- 55 | " | *Actinodaphne longifolia* Nakai | Baribarinoki | (Actinodaphne) | W |
| H- 55 | | | | | |
| H- 56 | Saxifraga-ceae | *Hydrangea paniculata* Sieb. | Noriutsugi | Panicle hydrangea | A |
| H- 56 | | | | | |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H- 57 | Hamameli-daceae | *Distylium racemosum* Sieb. et Zucc. | Isunoki | (Distylium) | W |
| H- 58 | " | *Hamamelis japonica* Sieb. et Zucc. | Mansaku | Japanese witch hazel | A |
| H- 59 | " | *Hamamalis japonica* Sieb. et Zucc. var. *obtusata* Matsum. | Marubamansaku | (Witch hazel) | K |
| H- 60 | Rosaceae | *Prunus sargenti* Rehd. | Oyamazakura | (Plum, Cherry) | W |
| H- 61 | " | *Prunus sargenti* Rehd. subsp. *jamasakura* Ohwi | Yamazakura | (Plum, Cherry) | W |
| H- 62 | " | *Prunus ssiori* Fr. Schm. | Shiurizakura | (Plum, Cherry) | W |
| H- 63 | " | *Prunus subhirtella* Miq. | Higanzakura | (Plum, Cherry) | W |
| H- 64 | " | *Prunus spinulosa* Sieb. et Zucc. | Rinboku | (Plum, Cherry) | W |
| H- 65 | " | *Eriobotrya japonica* Lindl. | Biwa | Loquat | K |
| H- 66 | " | *Chaenomeles sinensis* Koehne | Karin | (Flowering quince) | K |
| H- 67 | " | *Sorbus commixta* Hedl. | Nanakamado | Mountain ash | A |
| H- 68 | " | *Crataegus chlorosarca* Maxim. | Kuromisanzashi | (Hawthorn) | W |
| H- 69 | Leguminosae | *Albizzia julibrissin* Durazz. | Nemunoki | Silk flower | W |
| H- 70 | " | *Maackia amurensis* Rupe. et Maxim. var. *buergeri* C. K. Schneid. | Inuenju | (Maackia) | W |
| H- 71 | " | *Cladrastis platycarpa* Makino | Fujiki | (Yellow wood) | W |
| H- 72 | " | *Wisteria prachybotrys* Sieb. et Zucc. | Yamafuji | (Wisteria) | A |
| H- 73 | " | *Robinia pseudoacacia* L. | Harienju | Black locust | K |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H- 74 | Rutaceae | *Zanthoxyllum ailanthoides* Sieb. et Zucc. | Karasuzansho | (Toothache tree) | K |
| H- 75 | " | *Phellodendron amurense* Rupr. | Kihada | Amur cork tree | W |
| H- 76 | Simarouba-ceae | *Ailanthus altissima* Swingle | Niwaurushi | Tree of heaven | K |
| H- 77 | " | *Picrasma quessioides* Benn. | Nigaki | (Bitter wood) | W |
| H- 78 | Euphorbia-ceae | *Mallotus japonicus* Muel. Arg. | Akamegashiwa | (Mallotus) | A |
| H- 79 | " | *Daphniphyllum macropodium* Miq. | Yuzuriha | (Daphniphyllum) | W |
| H- 79 | | | | | |
| H- 80 | Anacardia-ceae | *Rhus succedanea* L. | Hazenoki | Japanese wax tree | W |
| H- 81 | " | *Rhus sylvestris* Sieb. et Zucc. | Yamahaze | (Sumac) | W |
| H- 82 | " | *Rhus verniciflua* Stokes | Urushi | Varnish tree | A |
| H- 82 | | | | | |
| H- 83 | Aquifolia-ceae | *Ilex crenata* Thunb. | Inutsuge | Japanese holly | W |
| H- 84 | " | *Ilex macropoda* Miq. | Aohada | (Holly) | W |
| H- 84 | | | | | |
| H- 85 | " | *Ilex rotunda* Thunb. | Kuroganemochi | (Holly) | W |
| H- 86 | Aceraceae | *Acer crataegifolium* Sieb. et Zucc. | Urikaede | Hawthorn maple | A |
| H- 87 | " | *Acer japonicum* Thunb. | Hauchiwakaede | Fullmoon maple | A |
| H- 88 | " | *Acer mono* Maxim. | Itayakaede | Painted maple | A |
| H- 89 | " | *Acer mono* Maxim. var. *mayrii* Koidz. | Beniitaya | (Maple) | A |
| H- 89 | | | | | |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H- 90 | Aceraceae | *Acer rufinerve* Sieb. et Zucc. | Urihadakaede | (Maple) | W |
| H- 91 | " | *Acer palmatum* Thunb. | Irohamomiji | (Maple) | W |
| H- 92 | Hippocast-anaceae | *Aesculus turbinata* Blume | Tochinoki | Japanese horse-chestnut | A |
| H- 93 | Sapindaceae | *Sapindus mukurosii* Gaerten. | Mukuroji | Soap nut tree | W |
| H- 94 | Sabiaceae | *Meliosma myriantha* Sieb. et Zucc. | Awabuki | (Meliosma) | A |
| H- 95 | " | *Meliosma oldhami* Miq. | Yanbaruawabuki | (Meliosma) | K |
| H- 96 | " | *Meliosma rigida* Sieb. et Zucc. | Yamabiwa | (Meliosma) | W |
| H- 97 | Rhamnaceae | *Hovenia dulcis* Thunb. | Kenponashi | Japanese raisin tree | A |
| H- 98 | Elaeocarpa-ceae | *Elaeocarpus japonicus* Sieb. et Zucc. | Kobanmochi | (Elaeocarpus) | W |
| H- 99 | Tiliaceae | *Tilia japonica* Simk. | Shinanoki | Shina-lime | W |
| H-100 | " | *Tilia kiusiana* Makino et Shirasawa | Heranoki | Kiushu-linden | W |
| H-101 | Actinidia-ceae | *Actinidia polygama* Maxim. | Matatabi | (Actinidia) | A |
| H-102 | Theaceae | *Camellia japonica* L. | Tsubaki | Camellia | W |
| H-103 | " | *Camellia sasanqua* Thunb. | Sazanka | (Camellia) | W |
| H-104 | " | *Stewartia monadelpha* Sieb. et Zucc. | Himeshara | (Stewartia) | W |
| H-105 | " | *Ternstroemia japonica* Thunb. | Mokkoku | (Ternstroemia) | W |
| H-106 | " | *Eurya japonica* Thunb. | Hisakaki | Japanese Eurya | W |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H-107 | Flacourtiaceae | *Idesia polycarpa* Maxim. | Iigiri | (Idesia) | W |
| H-108 | Araliaceae | *Aralia elata* Seem. | Taranoki | Japanese angelica tree | A |
| H-109 | " | *Acanthopanax sciadophylloides* Fr. et Sav. | Koshiabura | (Acanthopanax) | A |
| H-110 | " | *Evodiopanax innovans* Nakai | Takanotsume | (Evodiopanax) | A |
| H-111 | " | *Kalopanax septemlobus* Koidz. | Sen | Castor Aralia | W |
| H-112 | " | *Kalopanax semtemlobus* Koidz. var. *lutchuense* Nemoto | Miyakodara | (Castor Aralia) | W |
| H-113 | Cornaceae | *Cornus controversa* Hemsl. | Mizuki | Cornel | W |
| H-114 | " | *Cornus macrophylla* Wall. | Kumanomizuki | (Cornel) | W |
| H-115 | Clethraceae | *Clethra barbinervis* Sieb. et Zucc. | Ryobu | Clethra | A |
| H-116 | Ericaceae | *Rhododendron tashiroi* Maxim. | Sakuratsutsuji | (Rose bay) | W |
| H-117 | " | *Pieris japonica* D. Don | Asebi | Japanese Andromeda | A |
| H-118 | " | *Lyonia elliptica* Okuyama | Nejiki | (Lyonia) | A |
| H-119 | " | *Vaccinium bracteatum* Thunb. | Shashanpo | (Bilberry) | W |
| H-120 | Ebenaceae | *Diospyros kaki* Thunb. var. *sylvestris* Makino | Yamagaki | (Persimmon) | W |
| H-121 | " | *Diospyros morrisiana* Hance | Tokiwagaki | (Persimmon) | W |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H-122 | Symplocaceae | *Symplocos chinensis* Druce var. *leucocarpa* Ohwi f. *pilosa* Ohwi | Sawafutagi | Japanese turquoise tree | A |
| H-123 | " | *Symplocos lucida* Sieb. et Zucc. | Kuroki | (Sweetleaf) | W |
| H-124 | " | *Symplocos prunifolia* Sieb. et Zucc. | Kurobai | (Sweetleaf) | W |
| H-125 | Styraceae | *Styrax japonica* Sieb. et Zucc. | Egonoki | Japanese snowbell | W |
| H-126 | " | *Pterostyrax hipsida* Sieb. et Zucc. | Obaasagara | Fragrant eponlette | A |
| H-126 | | | | | |
| H-127 | Oleaceae | *Syringa reticulata* Hara | Hashidoi | (Lilac) | W |
| H-128 | " | *Ligustrum lucidum* Ait. | Tonezumimochi | Glossy Privet | K |
| H-129 | " | *Fraxinus languinosa* Koidz. var. *serrata* Nakai | Kobanotoneriko | (Ash) | W |
| H-129 | | | | | |
| H-130 | " | *Fraxinus mandschurica* Rupr. var. *japonica* Max. | Yachidamo | Japanese Manchurian ash | W |
| H-131 | " | *Fraxinus longicuspis* Sieb. et Zucc. | Yamatoaodamo | (Ash) | A |
| H-131 | | | | | |
| H-132 | " | *Fraxinus spaethiana* Lingelsh | Shioji | (Ash) | A |
| H-132 | | | | | |
| H-133 | " | *Fraxinus insularis* Hemsl. | Shimatoneriko | (Ash) | K |
| H-134 | Boraginaceae | *Ehretia ovalifolia* Hassk. | Chishanoki | (Ehretia) | W |
| H-134 | | | | | |
| H-135 | Verbenaceae | *Callicarpa japonica* Thunb. | Murasaki-shikibu | (Beauty berry) | A |
| H-136 | " | *Clerodendron trichotomum* Thunb. | Kusagi | (Clerodendron) | A |

Table 7. Hardwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| H-137 | Scrophulariaceae | *Paulownia tomentosa* Steud. | Kiri | Kiri | W |
| H-138 | Caprifoliaceae | *Sambucus racemosa* L. subsp. *sieboldiana* Hara | Niwatoko | Red-berried elder | A |

* Underlined figure represents a sapwood sample.

** According to Kitamura and Okamotos' "Coloured Illustrations of Trees and Shurubs of Japan" (Genshoku Nippon Jumoku Zukan), Hoikusha, Osaka and Tokyo, (1960).

*** Mainly according to Kishima, Okamoto and Hayashis' "Atlas of Wood in Colour" (Genshoku Mokuzai Dai Zukan), Hoikusha, Osaka and Tokyo, (1962), and occasionally to Barner's "Die Nuthölzer der Welt" (Neudruck), Banden 1-4, Verlag von J. Cramer, Weinheim, (1961-1962). The bracketed name is the general name of the genus.

**** The material was taken from the following sources:
A: Kyoto University Forests in Ashiu, Kyoto Pref., K: Kyoto Univeristy Forests in Kamigamo Experimental Station, Kyoto Pref., W: Collection of Wood Research Institute, Kyoto University.

| Resistance | | Loss of weight (%) |
|---|---|---|
| I | Very resistant | 0 to 5 |
| II | Resistant | 5 to 15 |
| III | Moderately resistant | 15 to 25 |
| IV | Non-resistant | 25 to 40 |
| V | Perishable | over 40 |

According to this scheme, decay resistance of temperate hardwood species is shown in Fig. 38 (P 92-P 95).

Thirty-two species were classified into class I and class II. Of these species, *Castanea crenata* (H-29)[89,134], *Zelkova serrata* (H-35)[89,134] and *Morus bombycis* (H-39)[89,96,134] are well known for their higher decay resistance. It is conceivable that presence of tannins and related compounds in *C. crenata*[64], keyakinin and keyakinol in *Z. serrata*[49], and oxyresveratol and resveratol in *M. bombycis*[80] is mainly responsible for the higher resistance of these species.

*Ternstroemia japonica* (H-105) is known as a very resistant species against termite attack[75]. It is recognized that the cause of the resistance is associated with the presence of saponins in the wood extractives[75,96,131], though the mechanism of the antitermitic action has not been elucidated satisfactorily. If saponins are also associated with the higher resistance of *T. japonica* against *C. globosum*, surface and hemolytic activities of saponins may play an important role in their antifungal action[60].

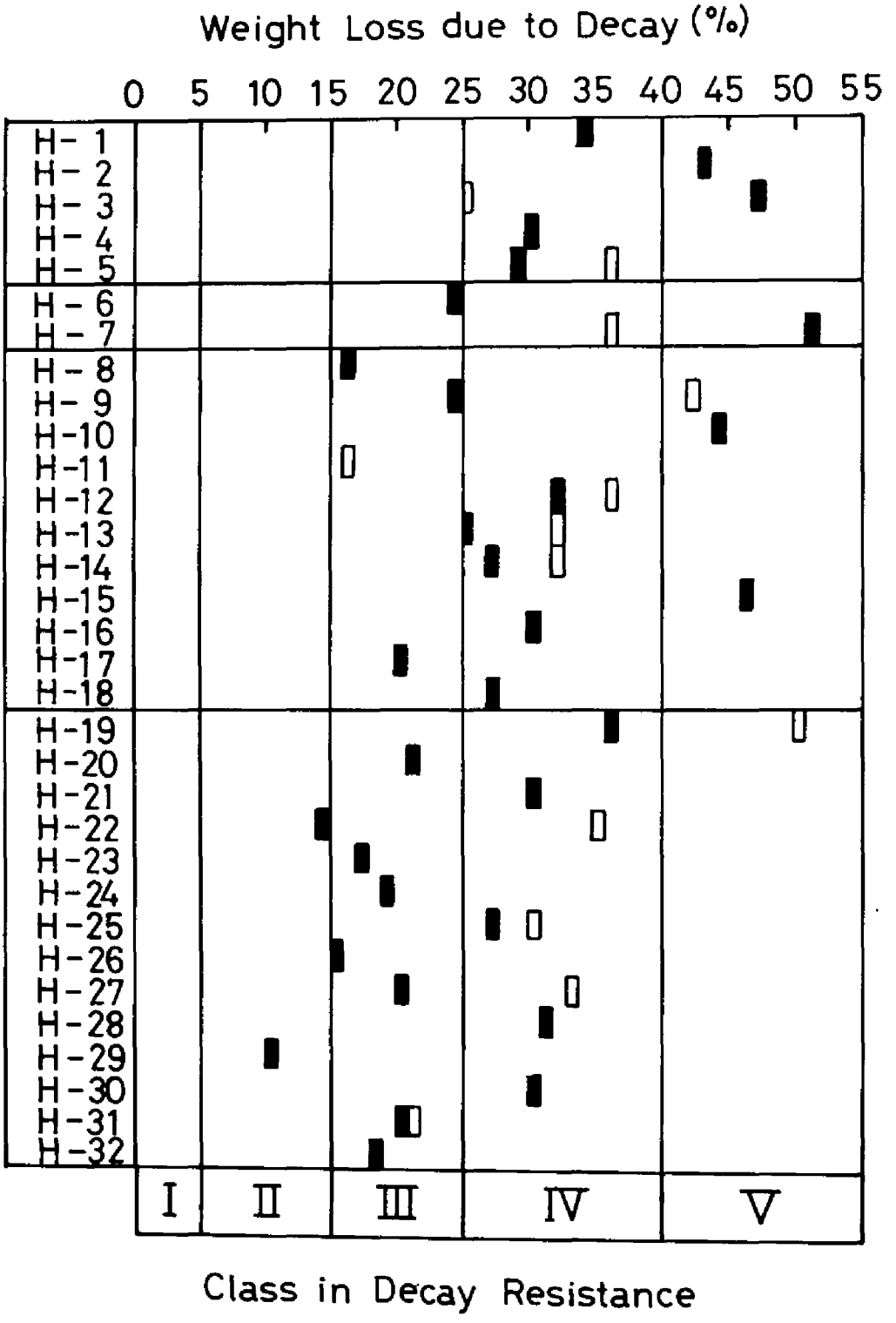
*Magnolia obovata* (H-47)[89], *Maackia amurensis* var. *buergeri* (H-70)

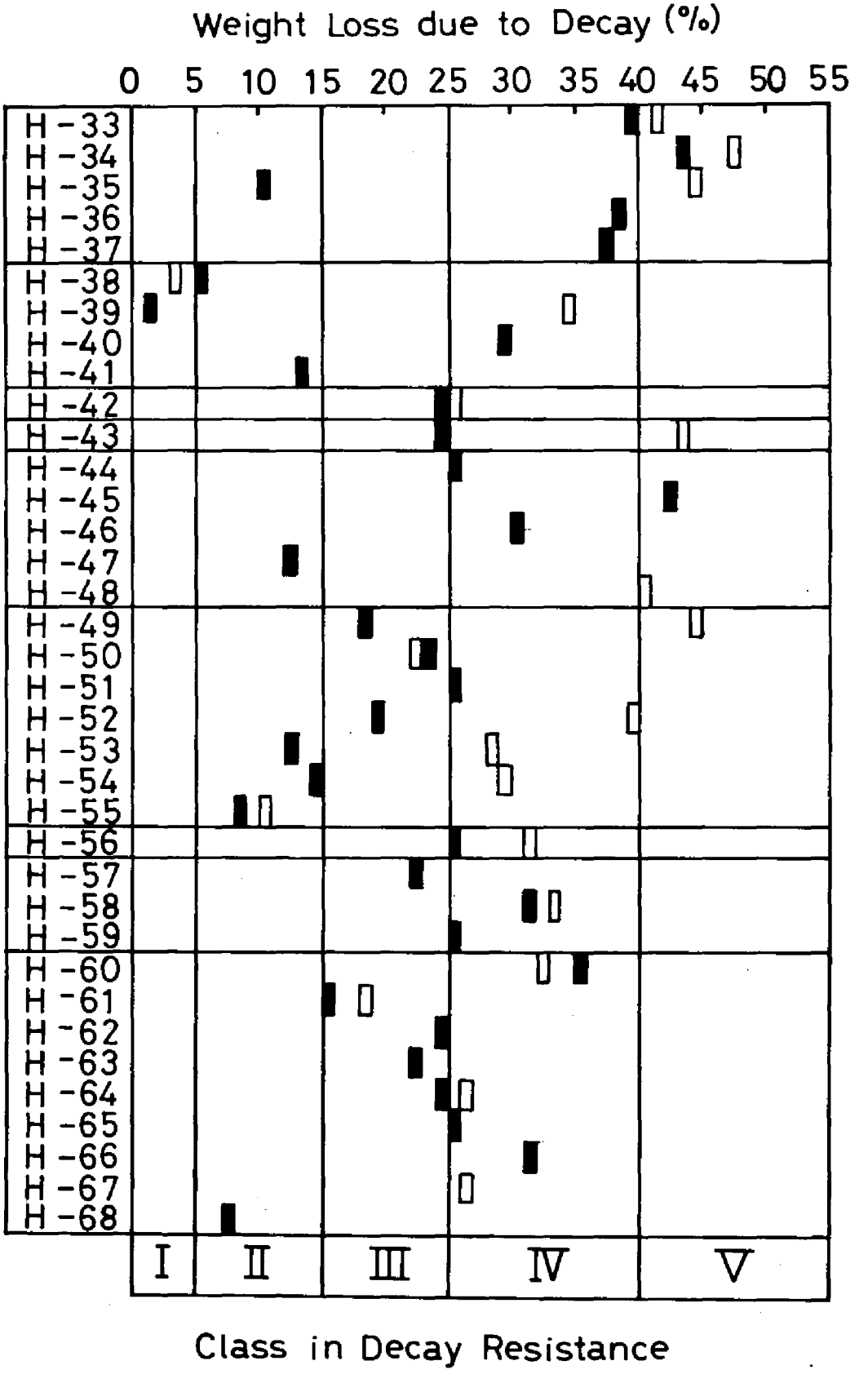
134), *Robinia pseudoacacia* (H-73)[89,134], *Rhus succedanea* (H-80)[131] and *Camellia japonica* (H-102)[75] have been also designated as resistant species. The correlation between resistance and extractives has not yet been established on these species, though various extractives have been identified, for example, alkaloids of *Magnolia*[127,128], fisetin and fustin of *Rhus*[59] and saponins of *Camellia*[52].

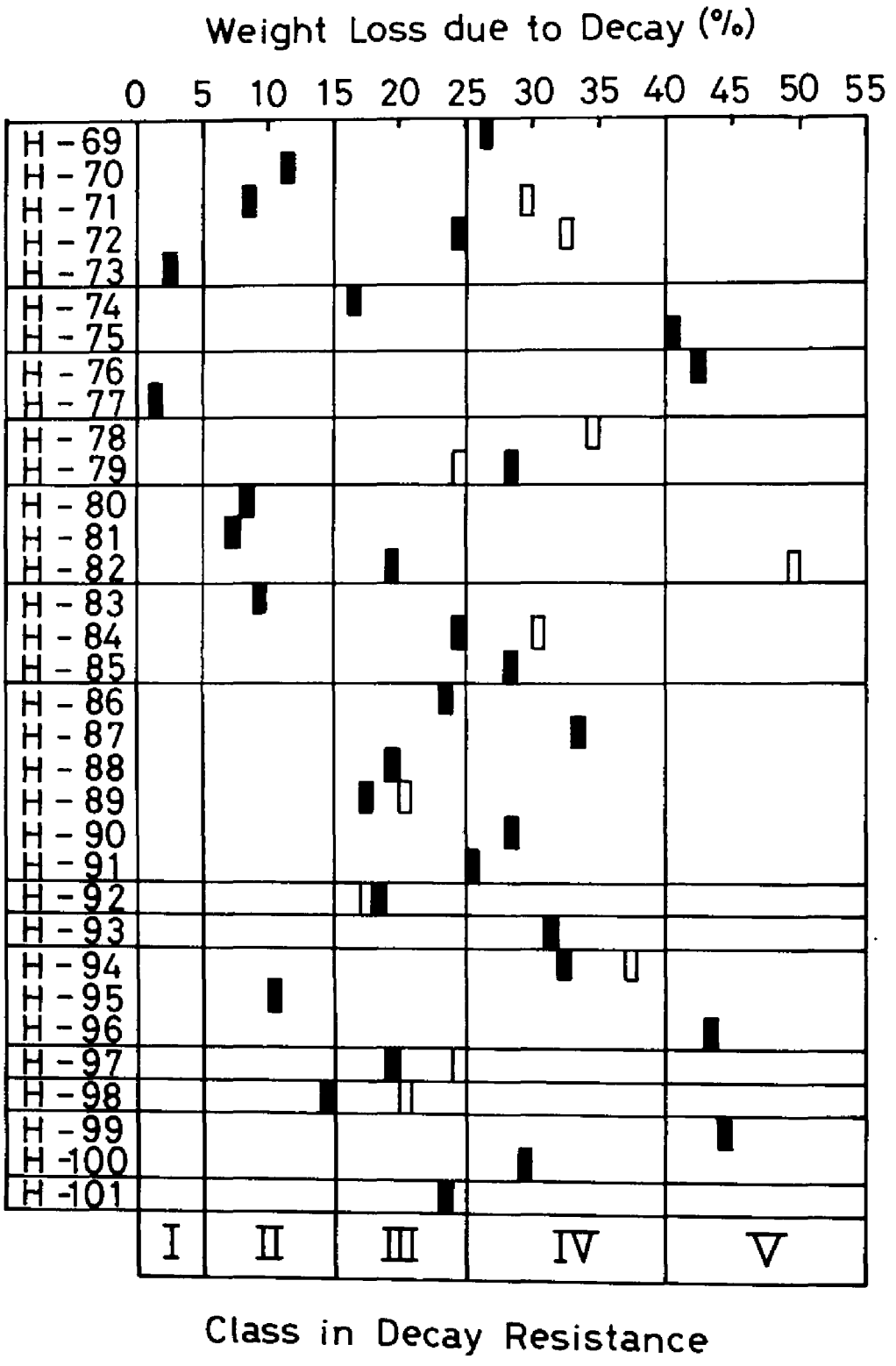
Information on the resistance of other species classified in class I and class II could not be obtained. However, oxyresveratol and resveratol have been isolated from *Cudrania tricuspidata* (H-41)[52] and *Morus alba* (H-38)[52]. Furansesquiterpene and paulownin may be partly responsible for the higher resistance in *Neolitsea aciculata* (H-53)[124] and *Paulownia tomentosa* (H-137)[113], respectively.

All Ericaceous species tested, i.e. *Rhododendron tashiroi* (H-116), *Pieris japonica* (H-117, 117), *Lyonia elliptica* (H-118) and *Vaccinium bracteatum* (H-119), were very resistant against *C. globosum*. Grayanotoxin, which is normally occurring in this family[125], may be partly associated with the higher resistance of these species. In addition, occurrence of pieristoxin in *P. japonica*[91], and of lyoniatoxin and lyoniol in *L. elliptica*[135,136] has been reported.

*Cercidiphyllum japonicum* (H-43)[89], *Cinnamomum camphora* (H-49)[134], *Distylium racemosum* (H-57)[134,136] and *Prunus sargenti* subsp. *jamasakura* (H-61)[134] were not so resistant in this experiment as expected from their reputed resistance. On the contrary, *Juglans mandschurica* subsp. *sieboldiana* (H-6)[134], *Alnus hirsuta* var. *sibirica* (H-17)[89], *Quercus serrata* (H-32)[89], *Passania edulis* (H-32)[89], *Aesculus turbinata* (H-92)[134]


Weight Loss due to Decay (%)
0 5 10 15 20 25 30 35 40 45 50 55
H-1
H-2
H-3
H-4
H-5
H-6
H-7
H-8
H-9
H-10
H-11
H-12
H-13
H-14
H-15
H-16
H-17
H-18
H-19
H-20
H-21
H-22
H-23
H-24
H-25
H-26
H-27
H-28
H-29
H-30
H-31
H-32
I
II
III
IV
V
Class in Decay Resistance


Weight Loss due to Decay (%)
0 5 10 15 20 25 30 35 40 45 50 55
H-33
H-34
H-35
H-36
H-37
H-38
H-39
H-40
H-41
H-42
H-43
H-44
H-45
H-46
H-47
H-48
H-49
H-50
H-51
H-52
H-53
H-54
H-55
H-56
H-57
H-58
H-59
H-60
H-61
H-62
H-63
H-64
H-65
H-66
H-67
H-68
I
II
III
IV
V
Class in Decay Resistance


Weight Loss due to Decay (%)
0 5 10 15 20 25 30 35 40 45 50 55
H - 69
H - 70
H - 71
H - 72
H - 73
H - 74
H - 75
H - 76
H - 77
H - 78
H - 79
H - 80
H - 81
H - 82
H - 83
H - 84
H - 85
H - 86
H - 87
H - 88
H - 89
H - 90
H - 91
H - 92
H - 93
H - 94
H - 95
H - 96
H - 97
H - 98
H - 99
H -100
H -101
I
II
III
IV
V
Class in Decay Resistance

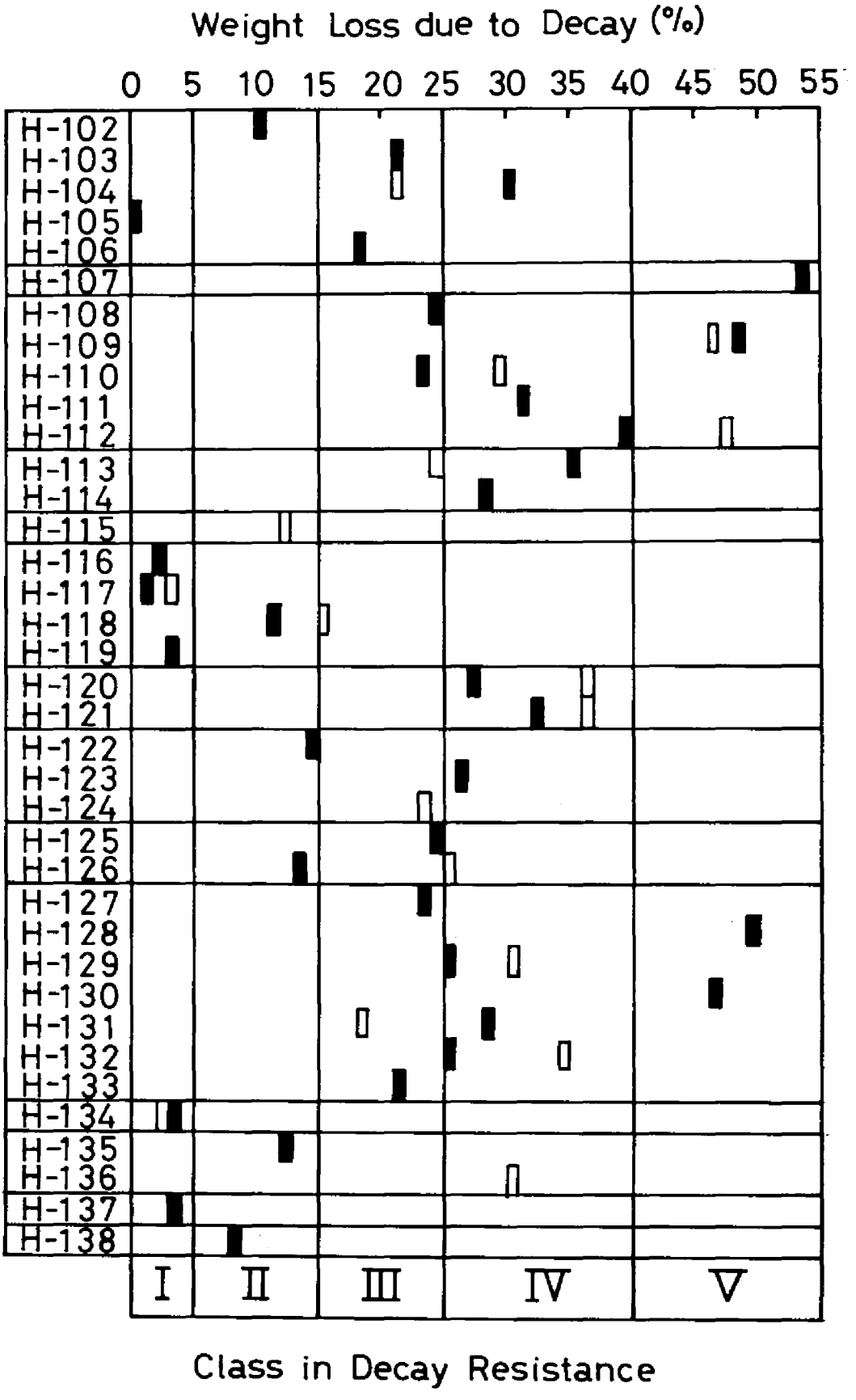

Weight Loss due to Decay (%)
0 5 10 15 20 25 30 35 40 45 50 55
H-102
H-103
H-104
H-105
H-106
H-107
H-108
H-109
H-110
H-111
H-112
H-113
H-114
H-115
H-116
H-117
H-118
H-119
H-120
H-121
H-122
H-123
H-124
H-125
H-126
H-127
H-128
H-129
H-130
H-131
H-132
H-133
H-134
H-135
H-136
H-137
H-138
I
II
III
IV
V
Class in Decay Resistance

Fig. 38. Decay resistance of each hardwood timber species against *Chaetomium globosum*. Number at leftmost corresponds with the number of timber species shown in Table 7. Black and white rectangles are illustrated as the weight loss value in heartwood and sapwood samples, respectively.

and *Elaeocarpus japonicus* (H-98)[75] were not so susceptible as expected.

Among species designated as non-resistant (class IV) and perishable (class V), *Populus maximowiczii* (H-1)[134], *Populus nigra* var. *italica* (H-2)[75], *Pterocarya rhoifolia* (H-7, 7)[89,134], *Betula platyphylla* var. *japonica* (H-14, 14)[75], *Fagus crenata* (H-19, 19)[134], *Castanopsis cuspidata* (H-30)[75,134], *Ulmus davidiana* var. *japonica* (H-33, 33)[134], *Cercidiphyllum japonicum* (H-43)[134], *Albizzia julibrissin* (H-69)[134], *Phellodendron amurense* (H-75)[134], *Tilia japonica* (H-99)[134], *Idesia polycarpa* (H-107)[75,89] and *Kalopanax septemlobus* (H-111)[75,89] have long been known as non-resistant or perishable species.

From the results obtained here, decay resistance of heartwood against *C. globosum* is classified on the family-base as follows:

Moraceae (excepting *Broussonetia*) and Ericaceae: very resistant,
Lauraceae and Leguminosae: resistant,
Fagaceae, Rosaceae and Aceraceae: moderately resistant to non-resistant,
Betulaceae, Araliaceae and Oleaceae: non-resistant,
Salicaceae and Ulmaceae (excepting *Zelkova*): perishable,
Magnoliaceae and Theaceae: variable in resistance.

Such variances of decay resistance among families and species do

not seem to be much different from those for Basidiomycotina found in the literature.

Table 8 shows the results with the effect of methanol extraction on decay resistance of heartwood samples of various hardwoods. Among 32 species of class I and class II, 25 species were tested. In addition, 4 species of class III, 1 species each of class IV and class V were tested for comparison. Unexpectedly, a positive effect (higher percent of weight loss in extracted blocks than that in non-extracted blocks) was significant in only five species (H-29, 39, 68, 77 and 137) of class I and class II. Such an effect was most pronounced in *Paulownia tomentosa* (H-137). A negative effect (lower percent of weight loss in extracted blocks) was found in nearly a half of species tested. A positive effect is possibly due to removal of toxic materials from wood. A non-significant effect found in resistant species may be attributed to; (1) insolubility of toxic materials in methanol, (2) insufficient removal of toxic materials soluble in methanol, and (3) presence of factors other than toxic materials. A negative effect in resistant species may be due to; (1) inhibiting action of methanol remaining in wood after extraction treatment, and (2) change in pH value caused by methanol extraction. A negative effect in less resistant species probably resulted mainly from a removal of nutrient substances during the extraction treatment.

Table 8. Effect of methanol extraction on the decay resistance of 31 temperate hardwood timber species against *Chaetomium globosum*.

| No.* | Content of methanol extractives (%) | Weight loss (%) | | Effect of extraction on decay** |
|---|---|---|---|---|
| | | Non-treated | Extracted with methanol | |
| H-105 | 1.48 | 0.63 | 1.41 | |
| H- 39 | 6.38 | 1.33 | 6.88 | + |
| H- 77 | 2.18 | 1.36 | 9.95 | + |
| H-117 | 1.73 | 1.80 | 0.20 | |
| H-116 | 2.08 | 2.41 | 0.07 | - |
| H-137 | 7.92 | 3.14 | 42.06 | + |
| H-119 | 3.10 | 3.58 | 2.27 | |
| H-134 | 0.85 | 3.69 | 2.89 | - |
| H- 38 | 4.77 | 5.63 | 6.42 | |
| H- 68 | 5.97 | 7.79 | 17.74 | + |
| H- 81 | 12.22 | 7.79 | 2.40 | - |
| H- 80 | 10.61 | 8.36 | 8.22 | |
| H- 55 | 0.77 | 8.69 | 5.34 | - |
| H- 71 | 4.83 | 8.69 | 7.82 | |
| H-138 | 0.90 | 8.94 | 3.18 | - |
| H- 83 | 2.72 | 9.92 | 5.61 | - |
| H- 35 | 10.98 | 10.18 | 9.07 | |
| H- 29 | 8.09 | 10.32 | 16.86 | + |
| H-102 | 0.64 | 10.84 | 8.86 | - |
| H-118 | 3.47 | 11.39 | 5.48 | - |
| H-135 | 4.09 | 12.12 | 7.13 | - |
| H- 47 | 2.00 | 12.44 | 25.26 | |
| H- 53 | 1.41 | 12.68 | 10.69 | |
| H- 41 | 0.67 | 13.53 | 6.72 | - |
| H- 98 | 10.00 | 14.60 | 7.82 | - |
| H- 74 | 1.46 | 16.63 | 8.80 | - |
| H- 82 | 5.02 | 19.64 | 10.45 | - |
| H-101 | 2.16 | 23.35 | 10.37 | - |
| H- 72 | 2.78 | 24.88 | 18.69 | |
| H- 19 | 1.02 | 36.63 | 32.47 | |
| H- 45 | 0.77 | 42.01 | 14.40 | - |

* See Table 7.

** Examined at 1 % level of significance by *t*-distribution.

## 3-2 Tropical (Southeast Asian) hardwoods[119)]

### Materials and Methods

The wood species used are listed in Table 9. Most of them are important species in their native countries. Sixty-four species covered 22 families, including 25 genera. Sampling of test blocks was made only from the heartwood portion which is regarded as commercially useful, since timbers with whole portion of heartwood were not obtained in this experiment. In addition to *C. globosum*, a white rot fungus, *Coriolus versicolor* Quél. (FES 1030), was used for comparison. Based on the results described in Chapter 1, the composition of the nutrient solution for *C. versicolor* was modified as follows:

$KH_2PO_4$ 3.0 g, $MgSO_4 \cdot 7H_2O$ 2.0 g, peptone 5.0 g, malt extract 10.0 g, glucose 25.0 g and distilled water 1000 ml.

The size of blocks, and procedures of decay tests and methanol extraction are the same as mentioned in 3-1.

### Results and Discussion

Weight loss in percent was not over 40 % for all the species used excepting T-18 and T-63. To facilitate the discussion, decay resistance was divided into four classes. Class I, II and III are based on the same scheme described in 3-1 but class IV represents the case in which the loss of weight is over 25 %. Tables 10 and 11 show the summarized results with the decay resistance of 64 species with specific gravity and content of methanol extractives. From Table 10 it can be seen that

Table 9. Tropical hardwood timber species used in the decay test.

| No. | Family | Botanical name* | Vernacular name** | Source*** |
|---|---|---|---|---|
| T- 1 | Fagaceae | *Castanopsis argentea* A. DC. | Saninten, Belangan saninten (In). | In |
| T- 2 | " | *Quercus* sp. | Pasang (In). | In |
| T- 3 | " | *Quercus* sp. | | Sab |
| T- 4 | Olacaceae | *Ochanostachys amentacea* Mast. | Petaling (In, Mly, Br), Petikal (Swk, Br), Tanggal (Sab), Amin, Lembasung, Pilung, Satan bagiuk, Tilokot, Tumbung asu (In). | In |
| T- 5 | " | *Scorodocarpus borneensis* Becc. | Kulim (Mly, In), Ungsumah (Swk), Bawan, Kasino, Madudu, Sinduk (In), Bawang hutan (Swk, Mly, Sab, Br), Bawing hutan (Br). | In |
| T- 6 | Lauraceae | *Eusideroxylon zwageri* Teysm. et Binn. | Belian (In, Sab, Swk), Uling, Ulin, Oelin, Onglen, Bulian, Bandjudjang, Talihan, Tihin, Tabulin (In), Tambulian (Ph). | Sab |
| T- 7 | " | *Eusideroxylon zwageri* Teysm. et Binn. | Ibid. | In |
| T- 8 | Hamamelidaceae | *Altingia excelsa* Noronha | Rasamala (In), Jutili (Ind). | In |
| T- 9 | Rosaceae | *Parinari oblongifolium* Hook. f. | Merbatu (In), Ampili, Karup daun pandjang, Katutukan, Ubah ubah leber daun (In), Membatu, Mentelor, Kemalau (Sab). | Sab |

Table 9. Tropical hardwood timber species used in the decay test (Continued).

| No. | Family | Botanical name* | Vernacular name** | Source*** |
|---|---|---|---|---|
| T-10 | Leguminosae | *Dalbergia latifolia* Roxb. | Sono keling, Angsana keling, Java palissandre (In), Rose-wood (Ind, Bma), East Indian rosewood (Ind), Bombay black-wood, Blackwood (Bma). | In |
| T-11 | " | *Intsia* sp. | Merbau (In, Mly, Sab), Kwila (NG), Lum-pho, Lumpaw, Makamong (Th). | In |
| T-12 | " | *Koompassia malaccensis* Maing. | Kempas (In, Mly, Swk), Empas (Sab), Impas (Sab, In), Pah, Mengeris, Upil (In). | Sin |
| T-13 | " | *Samanea saman* Merr. | Trembesi (In), Rain tree Acacia, Monkey pod. | In |
| T-14 | Burseraceae | *Canarium* sp. | | Sab |
| T-15 | " | *Santiria laevigata* Bl. | Kedondong (Mly, Br, Sab), Keranti lichin (Mly), Lalan, Longori, Peongga, Tapi tapi, Merambang (In). | Sab |
| T-16 | Euphorbia-ceae | *Neoscortechinia* sp. | | Sab |
| T-17 | Anacardia-ceae | *Mangifera* sp. | Bambangan (Sab). | Sab |
| T-18 | Aquifolia-ceae | *Ilex* sp. | | Sab |
| T-19 | Icacinaceae | *Cantleya corniculata* Becc. | Bedaru (In, Swk), Seranai (In), Dedaru (Mly). | In |

Table 9. Tropical hardwood timber species used in the decay test (Continued).

| No. | Family | Botanical name* | Vernacular name** | Source*** |
|---|---|---|---|---|
| T-20 | Sapindaceae | *Pometia pinnata* Forster | Kasai (Sab, Swk), Kasai daun besar (Mly), Malugai (Ph), Truong (Viet), Matoa, Taun (NG), Kasai besar daun, Kaseh, Kasie, Landoeng, Djampanga, Galunggung, Kempil kujat, Pangah, Singkuang (In). | Sab |
| T-21 | Tiliaceae | *Pentace* sp. | | Sab |
| T-22 | Theaceae | *Shima wallichii* Choisy | Puspa (In), Medang gatal (Sab, Mly), Ketinchi pudi (Br), Talo, Mangtan (Th), Gegata (Mly), | In |
| T-23 | Dipterocarpaceae | *Anisoptera costata* Korth. | Pengiran kesat, Mersawa daun lebar, Mersawa kesat, Kakan, Perapat hutan, Mersawa (Sab, Swk, Br, In). | Sab |
| T-24 | " | *Anisoptera* sp. | Mersawa (Sab, Swk, Br, In). | Sin |
| T-25 | " | *Balanocarpus heimii* King | Chengal, Penak (Mly), Takien chan (Th). | Sin |
| T-26 | " | *Cotylelobium* sp. | Giam (In), Resak (Mly). | In |
| T-27 | " | *Dipterocarpus confertus* V. Sl. | Keruing kobis (Sab, Swk, Br), Keruing tempurung (In). | Sab |
| T-28 | " | *Dipterocarpus grandiflorus* Blanco | Keruing belimbing (Sab, Mly), Keruing hidjau (In), Apitong (Ph). | Sab |
| T-29 | " | *Dipterocarpus* sp. | Keruing-(In). | In |
| T-30 | " | *Dipterocarpus* sp. | Keruing-(Mly). | Sin |
| T-31 | " | *Dryobalanopus aromatica* Gaertn. f. | Kapur (Mly), Kapur biasa (Sab), Kapur peringgi (Swk, Br), Kapur singkel (In). | Sin |

Table 9. Tropical hardwood timber species used in the decay test (Continued).

| No. | Family | Botanical name* | Vernacular name** | Source*** |
|---|---|---|---|---|
| T-32 | Dipterocarpaceae | *Dryobalanopus keithii* Sym. | Kapur gumbiat (Sab), Kalampait, Malampait, Santjulit, Tuali (In). | Sab |
| T-33 | " | *Hopea ferruginea* Parijs. | Selangan mata kuching (Sab), Luis merah (Swk, Br), Merawan dasal (In). | Sab |
| T-34 | " | *Hopea sangal* Korth. | Gagil (Sab), Merawan siput (Mly), Tjengal (In). | Sab |
| T-35 | " | *Parashorea* sp. | Urat mata-(sab). | Sab |
| T-36 | " | *Shorea fallax* W. Miejer | Seraya daun kasar (Sab). | Sab |
| T-37 | " | *Shorea gysbertsiana* Burck | Kawang jantong (Sab), Engkabang jantong (Swk, Br), Tengkawang telur (In). | Sab |
| T-38 | " | *Shorea leprosula* Miq. | Seraya tembaya (Sab), Meranti tembaya (Sab, In), Meranti tembaga (Mly, Swk, Br), Saya (Th). | Sab |
| T-39 | " | *Shorea macroptera* Dyer | Seraya melantai (Sab), Meranti melantai (Mly, Swk, Br), Saya (Th). | Sab |
| T-40 | " | *Shorea ovalis* Bl. | Seraya kepong (Sab), Meranti kepong (Mly, Swk, Br), Meranti kelungkung (In). | Sab |
| T-41 | " | *Shorea parvifolia* Dyer | Seraya punai (Sab), Meranti serang punai (Mly, In), Meranti samak (Swk, Br). | Sab |
| T-42 | " | *Shorea platycarpa* Heim | Seraya paya (Sab), Meranti paya (Mly, Swk, Br, In). | Sab |

Table 9. Tropical hardwood timber species used in the decay test (Continued).

| No. | Family | Botanical name* | Vernacular name** | Source*** |
|---|---|---|---|---|
| T-43 | Dipterocarpaceae | *Shorea* sp. | Meranti-(Mly) (Light red meranti) | Sin |
| T-44 | " | *Shorea* sp. | Meranti-(Mly) (Dark red meranti) | Sin |
| T-45 | " | *Shorea agami* Ashton | Melapi agama (Sab), Meranti puteh timbul, Badau pipit (Swk, Br). | Sab |
| T-46 | " | *Shorea symingtonii* Wood | Melapi bunga (Sab). | Sab |
| T-47 | " | *Shorea* sp. | Meranti-(Mly) (White meranti). | Sin |
| T-48 | " | *Shorea gibbosa* Brandis | Seraya kuning gajah (Sab), Lun gajah (Swk, Br), Damar hitam gajah (Mly), Damar buah (In). | Sab |
| T-49 | " | *Shorea hopeifolia* Sym. | Seraya kuning jantan (Sab), Lun jantan (Swk, Br), Damar kunjit, Maru kuning (In). | Sab |
| T-50 | " | *Shorea exelliptica* W. Meijer | Selangan batu tembaga (Swk, Br), Balau tembaga (Mly), Balau laut batu (In). | Sab |
| T-51 | " | *Shorea hypoleuca* W. Meijer | Selangan batu kelabu (Sab). | Sab |
| T-52 | " | *Shorea laevis* Ridl. | Selangan batu kumus (Sab, Swk, Br), Balau kumus (Mly), Balau tanduk (In). | Sab |
| T-53 | " | *Shorea leptoderma* W. Meijer | Selangan batu biabis (Sab). | Sab |
| T-54 | " | *Shorea* sp. | Balau-(In, Mly). | In |
| T-55 | " | *Shorea* sp. | Balau-(In, Mly). | Sin |
| T-56 | " | *Vatica micrantha* V. Sl. | Resak bulu (sab). | Sab |

Table 9. Tropical hardwood timber species used in the decay test (Continued).

| No. | Family | Botanical name* | Vernacular name** | Source*** |
|---|---|---|---|---|
| T-57 | Gonystylaceae | *Gonystylus* sp. | Ramin (Sab). | Sab |
| T-58 | Lythraceae | *Lagerstroemia speciosa* Pers. | Bungur, Langoti, Oindoloe (In), Bungor, Bungor ayer (Mly), Pinma (Bma), Banlang (Viet), Intanin (Th), Banaba (Ph), Jarul (Ind, Pak). | In |
| T-59 | Myrtaceae | *Metrosideros* sp. | Lara (In). | In |
| T-60 | Ebenaceae | *Diospyros macrophylla* Bl. | Kaju malam, Kaju hitam, Balam ayer, Bali, Tekam garam, Merpinang (Sab, Swk, Br), Kaju arang, Meribut, Siangus (Mly), Kaju arang siamang, Loting, Oela, Tonga, Banjan hitam, Itam, Kiling, Kising, Mitem, Salam bibit, Tulang tadung (In). | Sab |
| T-61 | " | *Diospyros* sp. | | In |
| T-62 | Loganiaceae | *Fagraea fragrans* Roxb. | Tembesu, Anrali, Kolahi, Nosoe (In), Tembesu padang (Mly, Br, Swk), Temasuk (Sab), Urung (Ph), Anan, Ananma, Burma yellow heart (Bma), Kankrao, Tetrau, Trai (Cam). | In |
| T-63 | Apocynaceae | *Alstonia* sp. | Pulai-(In, Mly). | Sab |
| T-64 | Rubiaceae | *Neonauclea bernardoi* Merr. | Bangka (Sab), Ludek (Ph). | Sab |

*, ** Mainly according to Sudo's "Tropical Woods" (Nanyozai), Chikyu Shuppan, Tokyo, (1970).
Bma: Burma, Br: Brunei, Cam: Cambodia, In: Indonesia, Ind: India, Mly: Malaya, NG: New Guinea, Pak: Pakistan, Ph: Philippine, Sab: Sabah, Swk: Sarawak, Th: Thailand, Viet: Vietnam.

*** Name of the organization which kindly offered the specimen.
In: Forest Products Research Institute, Bogor, Indonesia.
Sab: Conservator of Forests, Forestry Department, Sandakan, Sabah.
Sin: Trade Division, Ministry of Finance, Singapore.

the higher average specific gravity and the more amount of methanol extractives of the wood species result in the more resistant class in both cases of test fungi. As shown in Table 11, this tendency was statistically significant, and the relationship between specific gravity and decay resistance was most pronounced for *C. versicolor*.

When using *C. globosum*, there are more wood species belonging to class I and fewer in class IV than when using *C. versicolor*. It has been reported that decay by soft rot fungi, even under favorable conditions, occurs more gradually than that by Basidiomycotina does[6].

From the data obtained here, it may be possible to generalize that dense or extractive-rich species are more resistant to decay, though the more resistant species do not always fit into the rule. The specific gravity and the amount of extractives are frequently associated with each other reciprocally. High densities in *Balanocarpus heimii* (T-25), *Shorea laevis* (T-52) and *Vatica micrantha* (T-56) may be associated with their high content of methanol extractives (see Table 12), but dense and resistant species called "Selangan batu" or "Balau" do not contain so great a quantity of extractives (see T-50, 51, 53, 54 and 55 in Table 12), with the exception of T-52.

*Shorea* spp. have been usually divided into four groups, namely red meranti, white meranti, yellow meranti and Selangan batu, according to their specific gravity, hardness and color. T-36 to 44 belong to red meranti, T-45 to 47 to white meranti, T-48 and T-49 to yellow meranti, and T-50 to 55 to Selangan batu group. Fig. 39 shows the relationship between the percent of weight loss due to decay by *C. versicolor* and

Table 10. Summarized data for the decay resistance, specific gravity and content of methanol extractives of 64 tropical hardwood timber species.

| Fungus | *Chaetomium globosum* | | | | *Coriolus versicolor* | | | |
|---|---|---|---|---|---|---|---|---|
| Class in decay resistance* | I | II | III | IV | I | II | III | IV |
| Average specific gravity | 0.75 | 0.64 | 0.56 | 0.53 | 0.80 | 0.64 | 0.60 | 0.48 |
| Average content of methanol extractives (%) | 5.11 | 2.76 | 2.32 | 2.07 | 5.54 | 3.22 | 2.78 | 2.11 |
| Number of species | 24 | 18 | 19 | 3 | 20 | 15 | 18 | 11 |

* Weight loss due to decay, I: 0-5 %, II: 5-15 %, III: 15-25 %, IV: over 25 %.

Table 11. Statistical significance among four classes in decay resistance (see Table 10) of 64 tropical hardwood timber species in relation to the specific gravity and the content of methanol extractives.

| Fungus | | *Chaetomium globosum* | | | | *Coriolus versicolor* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Class* | I | II | III | IV | I | II | III | IV |
| Specific gravity | I | — | 2.381** | 4.153*** | 2.150** | — | 5.177*** | 5.674*** | 7.336*** |
| | II | 2.381** | — | 2.077** | 1.420 | 5.177*** | — | 0.878 | 3.592*** |
| | III | 4.153*** | 2.077** | — | 0.347 | 5.674*** | 0.878 | — | 2.637** |
| | IV | 2.150** | 1.420 | 0.347 | — | 7.336*** | 3.592*** | 2.637** | — |
| | Class* | I | II | III | IV | I | II | III | IV |
| Content of methanol extractives | I | — | 2.484** | 3.317*** | 1.468 | — | 2.338** | 3.153*** | 3.103*** |
| | II | 2.484** | — | 1.158 | 0.977 | 2.338** | — | 0.542 | 0.702 |
| | III | 3.317*** | 1.158 | — | 0.369 | 3.153*** | 0.542 | — | 1.684 |
| | IV | 1.468 | 0.977 | 0.369 | — | 3.103*** | 0.702 | 1.684 | — |

* Weight loss due to decay, I: 0-5 %, II: 5-15 %, III: 15-25 %, IV: over 25 %.

** Significant at 5 % level by $t$-distribution.

*** Significant at 1 % level by $t$-distribution.

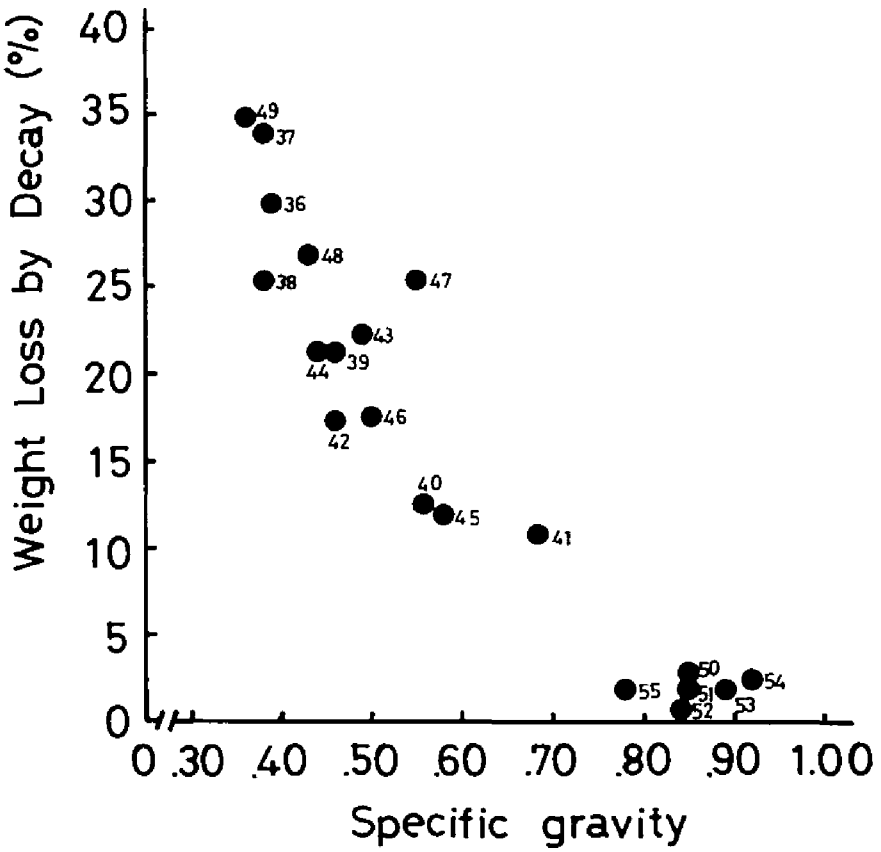



Fig. 39. Relationship between weight loss of *Shorea* spp. due to decay by *Coriolus versicolor* and their specific gravity (number on each plot corresponds with the number of timber species shown in Table 9).

original specific gravity of 20 *Shorea* species. It suggests that the correlation between decay resistance and specific gravity is highly significant in this case.

In Tables 12-15, specific gravity, content of methanol extractives, weight loss of non-extracted and extracted blocks due to decay, and the effect of methanol extraction on decay are given for each wood species.

Table 12. Weight loss, specific gravity and content of methanol extractives of tropical hardwood timber species in class I*

| No.** | Specific gravity | Content of methanol extractives (%) | *Chaetomium globosum* | | | *Coriolus versicolor* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| T- 4 | 0.66 | 4.19 | 1.38 | 1.43 | | 2.29 | 2.59 | |
| T- 5 | 0.68 | 1.32 | 0.30 | 0.14 | | 1.48 | 2.43 | + |
| T- 6 | 0.98 | 3.82 | 1.22 | 0.41 | | 1.04 | 0.60 | |
| T- 7 | 0.84 | 7.85 | 1.30 | 1.04 | | 0.94 | 0.46 | |
| T-10 | 0.71 | 9.19 | 1.20 | 11.78 | | 1.51 | 10.44 | + |
| T-11 | 0.67 | 11.47 | 2.02 | 2.21 | | 2.47 | 12.33 | + |
| T-19 | 0.98 | 2.64 | 2.03 | 4.52 | + | 2.77 | 2.60 | |
| T-22 | 0.52 | 1.44 | 0.61 | 1.75 | | | class III | |
| T-25 | 0.80 | 11.93 | 0.64 | 3.12 | + | 0.78 | 6.75 | + |
| T-26 | 0.77 | 10.26 | 0.18 | 3.03 | + | 0.41 | 8.39 | + |
| T-34 | 0.65 | 6.29 | 0.85 | 16.38 | + | 0.15 | 18.87 | + |
| T-39 | 0.46 | 1.96 | 3.06 | 1.00 | - | | class III | |
| T-40 | 0.56 | 2.33 | 2.89 | 3.17 | | | class II | |
| T-45 | 0.58 | 5.18 | 0.67 | 13.56 | + | | class II | |
| T-50 | 0.85 | 3.41 | 2.30 | 4.09 | | 3.04 | 1.49 | - |

Table 12. Weight loss, specific gravity and content of methanol extractives of tropical hardwood timber species in class I* (Continued).

| No.** | Specific gravity | Content of methanol extractives (%) | *Chaetomium globosum* | | | *Coriolus versicolor* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| T-51 | 0.85 | 3.05 | 1.30 | 1.79 | | 1.90 | 1.80 | |
| T-52 | 0.82 | 10.24 | 0.40 | 2.19 | + | 0.81 | 4.17 | |
| T-53 | 0.89 | 2.54 | 1.67 | 7.36 | + | 1.97 | 3.20 | |
| T-54 | 0.92 | 2.81 | 1.52 | 7.40 | + | 2.60 | 1.80 | - |
| T-55 | 0.78 | 3.53 | 2.18 | 4.03 | | 1.88 | 2.80 | + |
| T-56 | 0.84 | 8.17 | 1.87 | 23.76 | + | 4.14 | 8.69 | + |
| T-58 | 0.64 | 1.27 | 0.86 | 1.36 | | 1.47 | 0.37 | |
| T-59 | 0.85 | 1.72 | | class II | | 4.23 | 3.33 | |
| T-61 | 0.90 | 5.08 | 1.83 | 10.70 | + | 4.16 | 4.39 | |
| T-62 | 0.68 | 2.78 | 4.36 | 8.06 | + | | class II | |

* Weight loss due to decay, 0-5 %.

** See Table 9.

*** Examined at 1 % level of significance by *t*-distribution.

Table 13. Weight loss, specific gravity and content of methanol extractives of tropical hardwood timber species in class II*.

| No.** | Specific gravity | Content of methanol extractives (%) | *Chaetomium globosum* | | | *Coriolus versicolor* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| T- 1 | 0.62 | 6.46 | 7.31 | 20.67 | + | 11.17 | 22.43 | + |
| T- 2 | 0.70 | 2.35 | | class III | | 12.43 | 10.86 | |
| T- 3 | 0.79 | 1.05 | | class IV | | 14.27 | 11.84 | |
| T- 9 | 0.62 | 1.63 | 14.54 | 11.46 | | 8.44 | 9.66 | |
| T-12 | 0.69 | 1.57 | 13.87 | 10.55 | | | class III | |
| T-13 | 0.59 | 4.80 | 8.49 | 21.78 | + | 9.54 | 10.53 | |
| T-16 | 0.69 | 2.41 | 5.57 | 4.87 | - | 8.06 | 7.38 | |
| T-17 | 0.74 | 2.10 | 14.29 | 11.70 | | | class III | |
| T-21 | 0.57 | 1.09 | | class III | | 12.85 | 24.58 | + |
| T-23 | 0.52 | 2.79 | | class III | | 14.97 | 15.94 | |
| T-27 | 0.76 | 3.90 | 14.22 | 8.23 | - | | class III | |
| T-28 | 0.68 | 9.70 | 6.24 | 9.01 | | 11.49 | 12.21 | |
| T-31 | 0.66 | 3.68 | 5.21 | 4.90 | | | class III | |
| T-32 | 0.70 | 1.91 | 8.89 | 4.32 | - | | class III | |
| T-33 | 0.61 | 4.18 | 8.38 | 27.58 | + | | class III | |

Table 13. Weight loss, specific gravity and content of methanol extractives of tropical hardwood timber species in class II* (Continued).

| No.** | Specific gravity | Content of methanol extractives (%) | *Chaetomium globosum* | | | *Coriolus versicolor* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| T-40 | 0.56 | 2.33 | | class I | | 12.71 | 8.17 | - |
| T-41 | 0.68 | 2.21 | 10.18 | 13.62 | + | 11.09 | 8.02 | |
| T-42 | 0.46 | 1.47 | 11.61 | 17.01 | + | | class III | |
| T-43 | 0.49 | 2.90 | 8.51 | 5.83 | | | class III | |
| T-44 | 0.44 | 3.06 | 6.73 | 6.71 | | | class III | |
| T-45 | 0.58 | 5.18 | | class I | | 11.92 | 27.01 | + |
| T-47 | 0.55 | 3.80 | 8.35 | 11.97 | | | class IV | |
| T-57 | 0.64 | 1.81 | | class III | | 8.62 | 9.58 | |
| T-59 | 0.85 | 1.72 | 7.41 | 6.43 | | | class I | |
| T-62 | 0.68 | 2.78 | | class I | | 6.87 | 12.99 | |
| T-64 | 0.71 | 1.68 | 6.71 | 8.36 | | 14.97 | 11.74 | |

* Weight loss due to decay, 5-15 %.

** See Table 9.

*** Examined at 1 % level of significance by $t$-distribution.

Table 14. Weight loss, specific gravity and content of methanol extractives of tropical hardwood timber species in class III*.

| No.** | Specific gravity | Content of methanol extractives (%) | *Chaetomium globosum* | | | *Coriolus versicolor* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| T- 2 | 0.70 | 2.35 | 19.84 | 20.97 | | | class II | |
| T- 8 | 0.63 | 2.73 | 23.81 | 35.45 | | 24.52 | 35.79 | |
| T-12 | 0.69 | 1.57 | | class II | | 17.78 | 10.98 | |
| T-14 | 0.70 | 1.28 | 15.93 | 22.88 | | | class IV | |
| T-15 | 0.68 | 2.08 | 16.93 | 14.89 | | 22.98 | 10.33 | |
| T-17 | 0.74 | 2.10 | | class II | | 16.47 | 18.34 | |
| T-20 | 0.54 | 3.01 | 16.91 | 17.24 | | 16.04 | 12.22 | |
| T-21 | 0.57 | 1.09 | 16.28 | 14.84 | | | class II | |
| T-22 | 0.52 | 1.44 | | class I | | 18.67 | 18.34 | |
| T-23 | 0.52 | 2.79 | 19.51 | 26.12 | + | | class II | |
| T-24 | 0.50 | 3.24 | 21.62 | 28.95 | + | 18.42 | 45.12 | + |
| T-27 | 0.76 | 3.90 | | class II | | 17.76 | 13.16 | |
| T-29 | 0.60 | 3.16 | 21.16 | 28.42 | + | 20.14 | 29.12 | + |
| T-30 | 0.76 | 5.47 | 16.15 | 24.89 | + | 21.08 | 23.76 | |
| T-31 | 0.66 | 3.68 | | class II | | 20.39 | 16.54 | |
| T-32 | 0.70 | 1.91 | | class II | | 22.97 | 16.30 | - |
| T-33 | 0.61 | 4.18 | | class II | | 15.81 | 10.91 | |

Table 14. Weight loss, specific gravity and content of methanol extractives of tropical hardwood timber species in class III* (Continued).

| No.** | Specific gravity | Content of methanol extractives (%) | *Chaetomium globosum* | | | *Coriolus versicolor* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| T-35 | 0.59 | 0.50 | 24.93 | 29.42 | | | class IV | |
| T-36 | 0.39 | 1.34 | 16.26 | 15.93 | | | class IV | |
| T-37 | 0.38 | 1.72 | 21.15 | 13.79 | - | | class IV | |
| T-38 | 0.38 | 2.79 | 16.05 | 11.37 | - | | class IV | |
| T-39 | 0.46 | 1.96 | | class I | | 21.51 | 8.31 | - |
| T-42 | 0.46 | 1.47 | | class II | | 17.53 | 23.04 | |
| T-43 | 0.49 | 2.90 | | class II | | 22.35 | 30.43 | |
| T-44 | 0.44 | 3.06 | | class II | | 21.60 | 22.83 | |
| T-46 | 0.50 | 2.18 | 16.99 | 17.51 | | 17.68 | 29.34 | |
| T-49 | 0.36 | 3.30 | 22.56 | 34.04 | + | | class IV | |
| T-57 | 0.64 | 1.81 | 16.51 | 6.63 | - | | class II | |
| T-60 | 0.44 | 1.85 | 16.42 | 15.11 | | | class IV | |
| T-63 | 0.71 | 1.48 | 19.83 | 22.61 | | | class IV | |

* Weight loss due to decay, 15-25 %.

** See Table 9.

*** Examined at 1 % level of significance by *t*-distribution.

Table 15. Weight loss, specific gravity and content of methanol extractives of tropical hardwood timber species in class IV*.

| No.** | Specific gravity | Content of methanol extractives (%) | *Chaetomium globosum* | | | *Coriolus versicolor* | | |
|---|---|---|---|---|---|---|---|---|
| | | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| T- 3 | 0.79 | 1.05 | 30.98 | 30.31 | | | class II | |
| T-14 | 0.70 | 1.28 | | class III | | 29.07 | 7.64 | - |
| T-18 | 0.38 | 2.47 | 50.05 | 39.06 | - | 57.94 | 27.19 | - |
| T-35 | 0.59 | 0.50 | | class III | | 26.20 | 22.92 | |
| T-36 | 0.39 | 1.34 | | class III | | 29.96 | 30.63 | |
| T-37 | 0.38 | 1.72 | | class III | | 34.08 | 30.08 | |
| T-38 | 0.38 | 2.79 | | class III | | 25.54 | 12.38 | |
| T-47 | 0.55 | 3.80 | | class II | | 25.49 | 33.64 | |
| T-48 | 0.43 | 2.70 | 25.89 | 38.11 | + | 27.03 | 36.99 | + |
| T-49 | 0.36 | 3.30 | | class III | | 34.94 | 25.31 | |
| T-60 | 0.44 | 1.85 | | class III | | 30.48 | 18.41 | |
| T-63 | 0.71 | 1.48 | | class III | | 41.04 | 27.46 | |

* Weight loss due to decay, over 25 %.

** See Table 9.

*** Examined at 1 % level of significance by $t$-distribution.

As seen in Table 12, the majority of the very resistant species were common to both two test fungi; all species (with only one exception of T-59) assigned to class I by their decay resistance to *C. versicolor* were classified also in the same class in the case of *C. globosum*. Such a trend, however, did not hold for the other classes of decay resistance, reflecting the generally higher decaying ability of *C. versicolor* and the different preferences for wood species in the test fungi. Examination for significance in percent of weight loss in non-extracted blocks between the two test fungi indicated that only 6 species were more susceptible to *C. globosum* than to *C. versicolor*, and 27 species were more susceptible to *C. versicolor*, and the remaining 31 species were equally susceptible to both fungi. Particularly, *Quercus* spp. (T-2 and 3) and *Gonystylus* sp. (T-57) were more susceptible to *C. globosum*, and likewise T-22, 31, 32, 36, 37, 39, 40, 43, 44, 47, 60 and 63 were more susceptible to *C. versicolor*.

In contrast with the case of temperate hardwoods, a positive effect of methanol extraction was found in as many as 17 species and a negative effect only in 4 species among the 42 in class I and class II when tested with *C. globosum*. In the case of *C. versicolor*, a positive effect was found in 11 species and a negative effect in 3 species among the 35 in these classes. A positive effect common to both fungi occurred in only 10 species (T-2, 10, 24, 25, 26, 29, 34, 45, 48 and 56). This suggests that the two test fungi have different sensitivities to wood extractives. T-13, 23, 33, 49, 53, 54 and 60, in particular, showed a pronounced positive effect when tested with *C. globosum*, and T-11, 21 and 46, likewise,

showed a pronounced positive effect when tested with *C. versicolor*. T-14, 18, 39 and 57 showed a remarkable negative effect. A negative effect has often been observed in the extractive-poor and less resistant species such as beech wood (*Fagus crenata*). Consequently, it is possible to attribute such an effect to the removal of substances from wood blocks by methanol extraction, which facilitate the attack by wood-decaying fungi.

## 3-3 Softwoods[118)]

### Materials and Methods

The wood species used are listed in Table 16. Forty-five species of softwoods covered 9 families, including 25 genera. Heartwood samples were taken as near as possible from the intermediate portion. Sampling of sapwood samples was made also from the middle part. *C. globosum* and *C. versicolor* were used as test fungi. The size of blocks, composition of the nutrient solutions, and procedures of decay tests and methanol extraction were the same as described in 3-1.

### Results and Discussion

Decay resistance of 45 softwood species against *C. globosum* and *C. versicolor* is illustrated in Fig. 40. Very low decaying ability in *C. globosum* was confirmed here for various softwood species. Also *C. versicolor* could not cause severe weight loss of softwoods, as expected from its hardwood-preference. For the convenience of discussion, decay

Table 16. Softwood timber species used in the decay test.

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| S- 1 | Ginkgoaceae | *Ginkgo biloba* L. | Icho | Maidenhair tree | W |
| S- 2 | Taxaceae | *Taxus cuspidata* Sieb. et Zucc. | Ichii | Japanese yew | W |
| S- 3 | " | *Torreya nucifera* Sieb. et Zucc. | Kaya | Japanese Torreya | W |
| S- 4 | Podocarpaceae | *Podocarpus macrophylla* D. Don | Inumaki | Longleaf Podocarp | W |
| S- 5 | " | *Podocarpus nagi* Zoll et Moritz. | Nagi | Nagi Podocarp | W |
| S- 6 | Cephalotaxaceae | *Cephalotaxus harringtonia* K. Koch | Inugaya | Japanese plum yew | W |
| S- 7 | Araucariaceae | *Agathis alba* Foxw. | Agachisu | Agathis | S |
| S- 8 | Pinaceae (Abietoideae) | *Abies firma* Sieb. et Zucc. | Momi | Japanese fir | W |
| S- 9 | " | *Abies mariesii* Mast. | Aomoritodomatsu | Maries' fir | W |
| S-10 | " | *Abies sachalinensis* Fr. Schm. | Todomatsu | Sachalin fir | W |
| S-11 | " | *Abies sachalinensis* Fr. Schm. var. *mayriana* Miyabe et Kudo | Aotodomatsu | (Fir, Spruce) | W |
| S-12 | " | *Pseudotsuga japonica* Beissn. | Togasawara | Japanese Douglas fir | K |
| S-13 | " | *Tsuga sieboldii* Carr. | Tsuga | Japanese hemlock | W |
| S-14 | " | *Picea glehnii* Mast. | Akaezomatsu | Glehn's spruce | W |
| S-15 | " | *Picea jezoensis* Carr. | Ezomatsu | Yezo spruce | W |
| S-16 | " | *Picea abies* Karst. | Doitsutohi | Spruce | A |

Table 16. Softwood timber species used in the decay test (Continued).

| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| S-17 | Pinaceae (Abietoideae) | *Larix leptolepsis* Gord. | Karamatsu | Japanese larch | A |
| S-18 | " | *Larix gmelini* Ledeb. | Guimatsu | Kurile larch | W |
| S-19 | " | *Keteleeria davidiana* Beissn. | Yusan | Aburasugi | K |
| S-20 | (Pinoideae) | *Pinus densiflora* Sieb. et Zucc. | Akamatsu | Japanese red pine | W |
| S-21 | " | *Pinus pentaphylla* Mayr. | Himekomatsu | Japanese white pine | A |
| S-22 | " | *Pinus thunbergii* Parl. | Kuromatsu | Japanese black pine | W |
| S-23 | " | *Pinus tabulaeformis* Carr. | Manshukuromatsu | Manchurian pine | K |
| S-24 | " | *Pinus radiata* D. Don | Rajiatamatsu | Montrey pine | K |
| S-25 | " | *Pinus rigida* Mill. | Rigidamatsu | Black pine | K |
| S-26 | " | *Pinus taeda* L. | Tedamatsu | Torchpine | K |
| S-27 | " | *Pinus sylvestris* L. | Oshuakamatsu | Scots pine | K |
| S-28 | " | *Pinus nigra* Arn. | Oshukuromatsu | Corcican black pine | K |
| S-29 | " | *Pinus strobus* L. | Sutorobumatsu | White pine | K |
| S-30 | " | *Pinus virginiana* Mill. | Bajiniamatsu | Virginia pine | K |
| S-31 | " | *Pinus elliottii* Engelm. | Eriottimatsu | American pitch pine | K |
| S-32 | Sciadopityaceae | *Sciadopitys verticillata* Sieb. et Zucc. | Koyamaki | Umbrella pine | W |

Table 16. Softwood timber species used in the decay test (Continued).

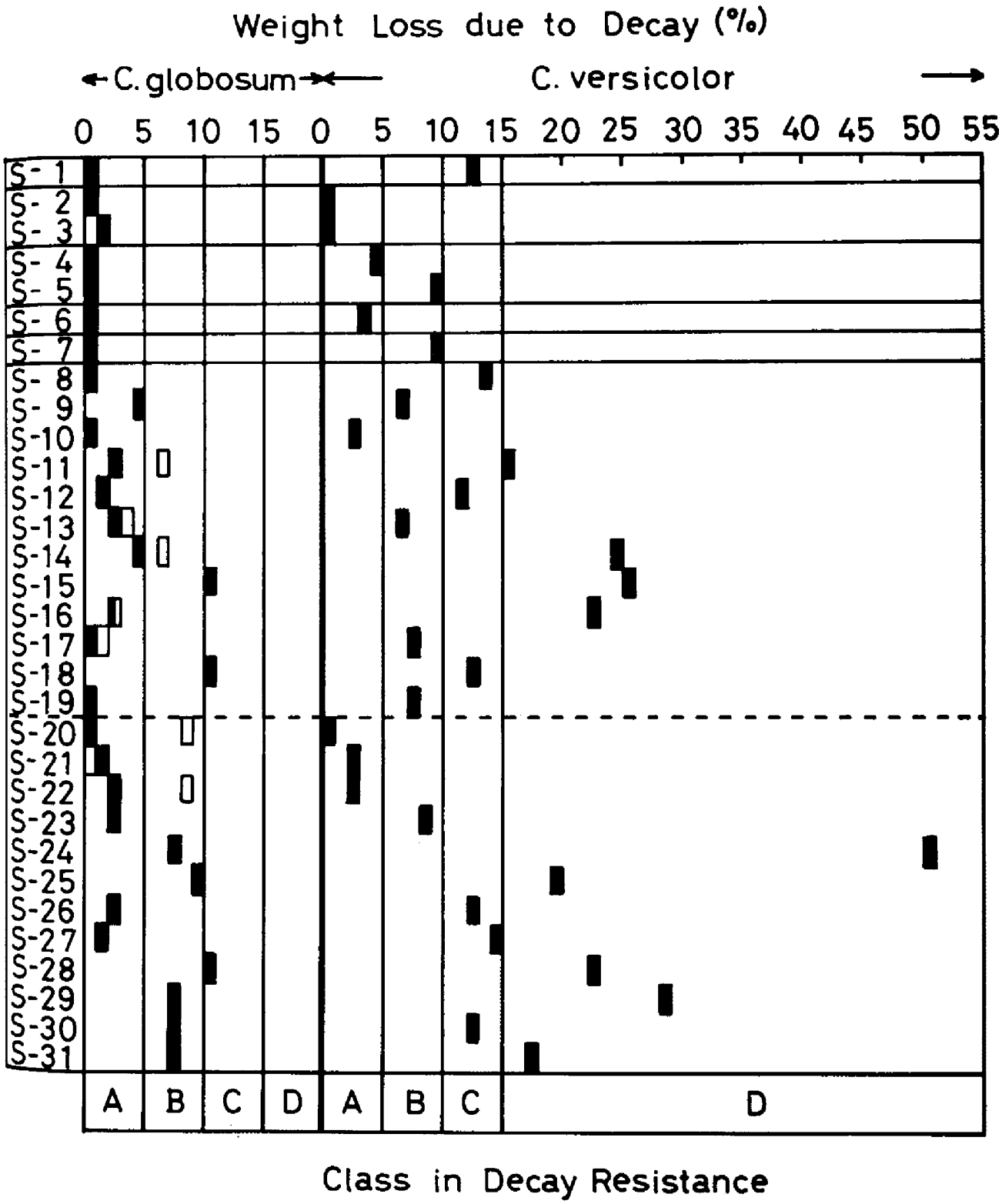
| No.* | Family | Botanical name** | Common name*** | | Source**** |
|---|---|---|---|---|---|
| | | | Japanese | English | |
| S-33 | Taxodiaceae | *Sequoia sempervirens* Endl. | Sekoia | Redwood | K |
| S-33 | | | | | |
| S-34 | " | *Metasequoia glyptostroboides* Hu et Cheng | Metasekoia | Dawn redwood | A |
| S-34 | | | | | |
| S-35 | " | *Glyptostrobus pensilis* K. Koch | Suisho | Swamp cypress | K |
| S-36 | " | *Taxodium distichum* Rich. | Numasugi | Bald cypress | K |
| S-37 | " | *Cryptomeria japonica* D. Don | Sugi | Japanese Cryptomeria | W |
| S-37 | | | | | |
| S-38 | " | *Cunninghamia konisii* Hayata | Randaisugi | Formosa fir | W |
| S-38 | | | | | |
| S-39 | " | *Taiwania cryptomerioides* Hayata | Taiwansugi | Taiwania | W |
| S-40 | Cupressaceae | *Chamaecyparis obtusa* Endl. | Hinoki | Japanese cypress | W |
| S-40 | | | | | |
| S-41 | " | *Chamaecyparis pisifera* Endl. | Sawara | Sawara cypress | W |
| S-41 | | | | | |
| S-42 | " | *Chamaecyparis formosensis* Matsum. | Benihi | Formosan cypress | W |
| S-42 | | | | | |
| S-43 | " | *Thuja standishii* Carr. | Nezuko | Japanese arbor-vitae | W |
| S-44 | " | *Thujopsis dolabrata* Sieb. et Zucc. | Asunaro | Hiba arbor-vitae | W |
| S-44 | | | | | |
| S-45 | " | *Juniperus virginiana* L. | Enpitsu-byakushin | Eastern red cedar | W |

* Underlined figure represents a sapwood sample.

** According to Kitamura and Okamotos' "Coloured Illustrations of Trees and Shurubs of Japan" (Genshoku Nippon Jumoku Zukan), Hoikusha, Osaka and Tokyo, (1960).

*** Mainly according to Kishima, Okamoto and Hayashis' "Atlas of Wood in Colour" (Genshoku Mokuzai Dai Zukan), Hoikusha, Osaka and Tokyo, (1962), and occasionally to Barner's "Die Nuthölzer der Welt" (neudruck), Banden 1-4, Verlag von J. Cramer, Weinheim (1961-1962). The bracketed name is the general name of the genus.

**** The material was taken from the following sources:
A: Kyoto University Forests in Ashiu, Kyoto Pref., K: Kyoto University Forests in Kamigamo Experimental Station, Kyoto Pref., S: Trade Division, Ministry of Finance, Singapore, W: Collection of Wood Research Institute, Kyoto University.


Weight Loss due to Decay (%)
← C. globosum →
← C. versicolor →
0 5 10 15 0 5 10 15 20 25 30 35 40 45 50 55
S- 1
S- 2
S- 3
S- 4
S- 5
S- 6
S- 7
S- 8
S- 9
S-10
S-11
S-12
S-13
S-14
S-15
S-16
S-17
S-18
S-19
S-20
S-21
S-22
S-23
S-24
S-25
S-26
S-27
S-28
S-29
S-30
S-31
A B C D A B C D
Class in Decay Resistance

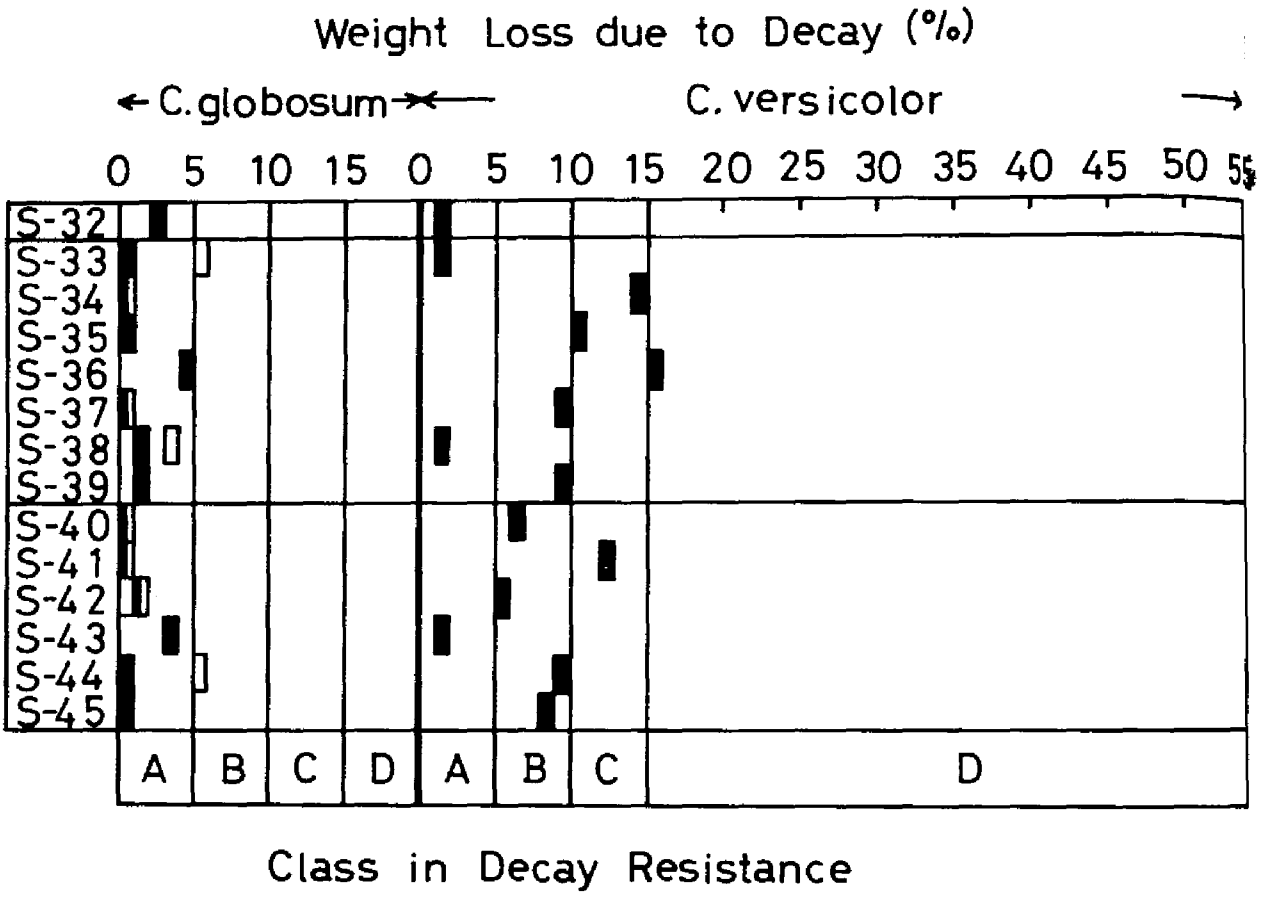



Fig. 40. Decay resistance of each softwood timber species against *Chaetomium globosum* and *Coriolus versicolor*. Number at left-most corresponds with the number of timber species shown in Table 16. Black and white rectangles are illustrated as the weight loss value in heartwood and sapwood samples, respectively.

resistance of softwoods was divided into four classes as follows:

A; loss of weight 0 to 5 (%),

B; loss of weight 5 to 10 (%),

C; loss of weight 10 to 15 (%),

D; loss of weight over 15 (%).

On the decay resistance of softwoods, it is generally accepted that most species in Pinaceae are more susceptible to fungal attack than those in Taxodiaceae and Cupressaceae. As seen in Fig. 40, such a tendency was apparent in both cases of *C. globosum* and *C. versicolor*, though the magnitudes of variance in decay resistance are not in the same order.

Effect of methanol extraction of heartwood samples of various softwoods is shown in Tables 17-20. Tables 21 and 22 show the summarized data for decay resistance and effect of methanol extraction related to content of methanol extractives in wood. A positive effect was significant in *Abies sachalinensis* (S-10), *Pinus densiflora* (S-20) and *Pinus thunbergii* (S-22) in both cases of *C. globosum* and *C. versicolor*. In *P. densiflora*, a positive effect was observed again, when tested with other wood-decaying fungi as shown below[120]:

| Fungus | Loss of weight (%) | |
|---|---|---|
| | Non-treated | Methanol-treated |
| *Coniophora puteana* | 3.02 | 26.33 |
| *Serpula lacrymans* | 9.81 | 40.55 |
| *Ganoderma lucidum* | 2.62 | 8.06 |
| *Lenzites betulina* | 2.82 | 17.67 |

As is evident from Table 22, frequencies of positive effect were equal in both cases of *C. globosum* and *C. versicolor*. However, positive effect common to both fungi occurred in above-described 3 species only. In the case of *C. globosum*, a positive effect was found mainly in the species of Pinaceae (10 species among the 13), and was not found in those of Taxodiaceae and Cupressaceae. In the case of *C. versicolor*, a posi-

Table 17. Effect of methanol extraction on the decay resistance of softwood timber species against *Chaetomium globosum* and *Coriolus versicolor* in class A*.

| No.** | Content of methanol extractives (%) | *C. globosum* | | | *C. versicolor* | | |
|---|---|---|---|---|---|---|---|
| | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| S- 1 | 1.36 | 0.68 | 2.08 | + | | class C | |
| S- 2 | 13.34 | 0.51 | 0.04 | | 0.00 | 7.34 | + |
| S- 3 | 5.21 | 1.94 | 0.00 | | 0.51 | 6.52 | + |
| S- 4 | 1.45 | 0.00 | 2.01 | + | 4.92 | 6.10 | |
| S- 5 | 1.39 | 0.10 | 0.89 | | | class B | |
| S- 6 | 1.01 | 0.68 | 3.14 | + | 3.52 | 4.43 | |
| S- 7 | 1.55 | 1.09 | 0.15 | | | class B | |
| S- 8 | 1.12 | 0.94 | 1.31 | | | class C | |
| S- 9 | 2.76 | 4.37 | 1.72 | - | | class B | |
| S-10 | 7.47 | 0.59 | 5.81 | + | 2.09 | 21.23 | + |
| S-11 | 2.76 | 2.61 | 4.81 | | | class D | |
| S-12 | 1.11 | 1.46 | 0.00 | | | class C | |
| S-13 | 3.88 | 2.87 | 2.10 | | | class B | |
| S-14 | 3.13 | 4.12 | 3.24 | | | class D | |
| S-16 | 1.19 | 2.83 | 8.34 | + | | class D | |
| S-17 | 1.83 | 0.82 | 3.14 | + | | class B | |
| S-19 | 0.93 | 0.19 | 0.53 | | | class B | |

Table 17. Effect of methanol extraction on the decay resistance of softwood timber species against *Chaetomium globosum* and *Coriolus versicolor* in class A* (Continued).

| No.** | Content of methanol extractives (%) | *C. globosum* | | | *C. versicolor* | | |
|---|---|---|---|---|---|---|---|
| | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| S-20 | 8.68 | 0.00 | 6.45 | + | 0.00 | 14.48 | + |
| S-21 | 7.72 | 1.64 | 3.00 | | 2.93 | 17.38 | + |
| S-22 | 7.17 | 2.02 | 5.53 | + | 2.81 | 9.59 | + |
| S-23 | 1.58 | 2.27 | 2.37 | | | class B | |
| S-26 | 1.16 | 2.37 | 0.34 | | | class C | |
| S-27 | 1.67 | 1.22 | 3.69 | + | | class C | |
| S-32 | 8.54 | 2.41 | 0.15 | - | 1.35 | 10.08 | + |
| S-33 | 10.18 | 0.06 | 0.00 | | 1.19 | 11.23 | + |
| S-34 | 4.28 | 0.03 | 0.00 | | | class C | |
| S-35 | 4.48 | 0.21 | 0.00 | | | class C | |
| S-36 | 0.83 | 4.92 | 4.22 | | | class D | |
| S-37 | 6.68 | 0.00 | 1.44 | | | class B | |
| S-38 | 6.05 | 1.24 | 0.84 | | 1.63 | 7.57 | + |
| S-39 | 10.72 | 1.84 | 0.13 | | | class B | |
| S-40 | 5.32 | 0.31 | 0.00 | | | class B | |
| S-41 | 6.97 | 0.87 | 0.03 | | | class C | |
| S-42 | 4.52 | 1.59 | 0.00 | | | class B | |

Table 17. Effect of methanol extraction on the decay resistance of softwood timber species against *Chaetomium globosum* and *Coriolus versicolor* in class A* (Continued).

| No.** | Content of methanol extractives (%) | *C. globosum* | | | *C. versicolor* | | |
|---|---|---|---|---|---|---|---|
| | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| S-43 | 9.03 | 3.14 | 1.54 | - | 1.09 | 9.49 | + |
| S-44 | 3.36 | 0.00 | 0.06 | | | class B | |
| S-45 | 2.48 | 0.00 | 0.14 | | | class B | |
| Ave. | — | 1.43 | 1.87 | — | 1.84 | 10.45 | — |

* Weight loss due to decay, 0-5 %.

** See Table 16.

*** Examined at 1 % level of significance by $t$-distribution.

Table 18. Effect of methanol extraction on the decay resistance of softwood timber species against *Chaetomium globosum* and *Coriolus versicolor* in class B*.

| No.** | Content of methanol extractives (%) | *C. globosum* | | | *C. versicolor* | | |
|---|---|---|---|---|---|---|---|
| | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| S- 5 | 1.39 | | class A | | 9.46 | 11.50 | |
| S- 7 | 1.55 | | class A | | 8.48 | 22.91 | + |
| S- 9 | 2.76 | | class A | | 6.81 | 11.98 | |
| S-13 | 3.88 | | class A | | 6.80 | 16.29 | + |
| S-17 | 1.83 | | class A | | 7.06 | 9.71 | |
| S-19 | 0.93 | | class A | | 7.22 | 9.43 | |
| S-23 | 1.58 | | class A | | 8.58 | 15.83 | |
| S-24 | 1.35 | 7.30 | 6.60 | | | class D | |
| S-25 | 0.97 | 9.00 | 17.00 | + | | class D | |
| S-29 | 2.21 | 7.34 | 9.97 | | | class D | |
| S-30 | 1.39 | 7.94 | 2.87 | - | | class C | |
| S-31 | 0.90 | 7.39 | 16.00 | + | | class D | |
| S-37 | 6.68 | | class A | | 9.50 | 9.42 | |
| S-39 | 10.72 | | class A | | 9.71 | 11.88 | |
| S-40 | 5.32 | | class A | | 6.11 | 18.67 | + |
| S-42 | 4.52 | | class A | | 5.91 | 9.17 | |
| S-44 | 3.36 | | class A | | 9.87 | 16.86 | |
| S-45 | 2.48 | | class A | | 8.62 | 10.74 | |
| Ave. | — | 7.79 | 10.49 | — | 8.01 | 13.41 | — |

* Weight loss due to decay, 5-10 %.

** See Table 16.

*** Examined at 1 % level of significance by *t*-distribution.

Table 19. Effect of methanol extraction on the decay resistance of softwood timber species against *Chaetomium globosum* and *Coriolus versicolor* in class C*.

| No.** | Content of methanol extractives (%) | *C. globosum* | | | *C. versicolor* | | |
|---|---|---|---|---|---|---|---|
| | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| S- 1 | 1.36 | | class A | | 12.94 | 7.02 | |
| S- 8 | 1.12 | | class A | | 13.76 | 10.64 | |
| S-12 | 1.11 | | class A | | 11.48 | 9.35 | |
| S-15 | 1.68 | 10.95 | 11.99 | | | class D | |
| S-18 | 2.35 | 10.54 | 16.27 | + | 12.16 | 12.77 | |
| S-26 | 1.16 | | class A | | 12.59 | 21.03 | |
| S-27 | 1.67 | | class A | | 14.98 | 24.02 | |
| S-28 | 1.52 | 10.20 | 24.80 | + | | class D | |
| S-30 | 1.39 | | class B | | 12.94 | 20.46 | |
| S-34 | 4.28 | | class A | | 14.11 | 16.35 | |
| S-35 | 4.48 | | class A | | 10.85 | 13.92 | |
| S-41 | 6.97 | | class A | | 12.08 | 20.64 | + |
| Ave. | — | 10.56 | 17.69 | — | 12.82 | 15.62 | — |

* Weight loss due to decay, 10-15 %.

** See Table 16.

*** Examined at 1 % level of significance by *t*-distribution.

Table 20. Effect of methanol extraction on the decay resistance of softwood timber species against *Chaetomium globosum* and *Coriolus versicolor* in class D*.

| No.** | Content of methanol extractives (%) | *C. globosum* | | | *C. versicolor* | | |
|---|---|---|---|---|---|---|---|
| | | Weight loss (%) | | Effect of extraction on decay*** | Weight loss (%) | | Effect of extraction on decay*** |
| | | Non-treated | Extracted with methanol | | Non-treated | Extracted with methanol | |
| S-11 | 2.76 | | class A | | 15.84 | 19.67 | |
| S-14 | 3.13 | | class A | | 24.89 | 18.36 | |
| S-15 | 1.68 | | class C | | 25.07 | 30.56 | |
| S-16 | 1.19 | | class A | | 22.87 | 16.21 | |
| S-24 | 1.35 | | class B | | 50.53 | 46.05 | |
| S-25 | 0.97 | | class B | | 19.16 | 12.35 | |
| S-28 | 1.52 | | class C | | 22.38 | 9.98 | - |
| S-29 | 2.21 | | class B | | 28.44 | 15.73 | |
| S-31 | 0.90 | | class B | | 17.02 | 14.88 | |
| S-36 | 0.83 | | class A | | 15.42 | 14.77 | |
| Ave. | — | — | — | — | 24.16 | 19.86 | — |

* Weight loss due to decay, over 15 %.

** See Table 16.

*** Examined at 1 % level of significance by *t*-distribution.

tive effect occurred scattering in several families. Different effects of extraction in a species were found in a few species (S-28, 32 and 43). Such a phenomenon was pronounced in *Pinus nigra* (S-28). Different sensitivities to wood extractives for the two test fungi were found in tropical woods, too (see 3-2).

As shown in Table 17, average weight loss values of non-treated and treated blocks were 1.43 and 1.87 (%) in the case of *C. globosum*, and 1.84 and 10.45 (%) in *C. versicolor*, respectively. This suggests that when decayed by *C. versicolor* methanol extraction was more significant for species in class A than decayed by *C. globosum*.

Average content of methanol extractives in the species in class A was higher than that of other classes in the case of *C. versicolor* (Table 21). Such a trend was verified statistically as shown in Table 23. Furthermore, average content of methanol extractives in the species which showed a significant positive effect was higher than others (Table 22). This was also confirmed statistically (Table 23). Consequently, in the case of softwoods as well as tropical hardwoods, it may be concluded that extractive-rich species are more resistant against *C. versicolor* and that the greater part of such species become less resistant after methanol extraction. However, such tendency did not hold exactly for *C. globosum* in both cases of hardwoods and softwoods. From the results obtained here, it can be concluded that the role of wood extractives is generally insignificant for softwoods which show the higher resistance against soft rot fungi.

Table 21. Summarized data for the decay resistance of 45 softwood timber species against *Chaetomium globosum* and *Coriolus versicolor*.

| Fungus | *C. globosum* | | | | *C. versicolor* | | | |
|---|---|---|---|---|---|---|---|---|
| Class in decay resistance* | A | B | C | D | A | B | C | D |
| Number of species | 37 | 5 | 3 | 0 | 12 | 13 | 10 | 10 |
| Pinaceae | 16 | 5 | 3 | 0 | 4 | 5 | 6 | 9 |
| (Abietoideae) | (10) | (0) | (2) | (0) | (1) | (4) | (3) | (4) |
| (Pinoideae) | (6) | (5) | (1) | (0) | (3) | (1) | (3) | (5) |
| Taxodiaceae | 7 | 0 | 0 | 0 | 2 | 2 | 2 | 1 |
| Cupressaceae | 6 | 0 | 0 | 0 | 1 | 4 | 1 | 0 |
| Others | 8 | 0 | 0 | 0 | 5 | 2 | 1 | 0 |
| Average content of methanol extractives (%) | 4.40 | 1.36 | 1.85 | — | 7.15 | 3.62 | 2.59 | 1.65 |

* Weight loss due to decay, A: 0-5 %, B: 5-10 %, C: 10-15 %, D: over 15 %.

Table 22. Summarized data for the effect of methanol extraction on the decay resistance of 45 softwood timber species against *C. globosum* and *Coriolus versicolor*.

| Fungus | *C. globosum* | | | *C. versicolor* | | |
|---|---|---|---|---|---|---|
| Effect of extraction on decay* | + | ± | - | + | ± | - |
| Number of species | 13 | 28 | 4 | 14 | 30 | 1 |
| Pinaceae | 10 | 12 | 2 | 5 | 18 | 1 |
| (Abietoideae) | (4) | (7) | (1) | (2) | (10) | (0) |
| (Pinoideae) | (6) | (5) | (1) | (3) | (8) | (1) |
| Taxodiaceae | 0 | 7 | 0 | 2 | 5 | 0 |
| Cupressaceae | 0 | 5 | 1 | 3 | 3 | 0 |
| Others | 3 | 4 | 1 | 4 | 4 | 0 |
| Average content of methanol extractives (%) | 2.89 | 4.10 | 5.43 | 7.22 | 2.31 | 1.52 |

* Examined at 1 % level of significance by $t$-distribution.

Table 23. Statistical significance among four classes in decay resistance (see Table 21) and effect of methanol extraction (see Table 22) in relation to the content of methanol extractives in 45 softwood timber species exposed to *Coriolus versicolor*.

| | Class* | A | B | C | D |
|---|---|---|---|---|---|
| Class in decay resistance | A | — | 2.553** | 3.688*** | 4.914*** |
| | B | 2.553*** | — | 1.141 | 2.348** |
| | C | 3.688*** | 1.141 | — | 1.382 |
| | D | 4.914*** | 2.348** | 1.382 | — |
| | Effect | + | ± | | - |
| Effect of extraction on decay | + | — | 7.337*** | | 2.425** |
| | ± | 7.337*** | — | | 0.372 |
| | - | 2.425** | 0.372 | | — |

* Weight loss due to decay, A: 0-5 %, B: 5-10 %, C: 10-15 %, D: over 15 %.

** Significant at 5 % level by $t$-distribution.

*** Significant at 1 % level by $t$-distribution.

## 3-4 Summary

Decay resistance of various wood species (138 spp. of temperate hardwoods, 64 spp. of tropical hardwoods and 45 spp. of softwoods) against a soft rot fungus, *Chaetomium globosum* Kunze, was estimated with reference to methanol extractives in wood by laboratory sand-block tests. For comparison, a white rot fungus, *Coriolus versicolor* Quél., was used in tests for tropical hardwoods and softwoods.

*Morus alba*, *Morus bombycis*, *Robinia pseudoacacia*, *Picrasma quassioides*, *Ternstroemia japonica*, *Rhododendron tashiroi*, *Pieris japonica*, *Vaccinium bracteatum*, *Ehretia ovalifolia* and *Paulownia tomentosa* were designated as very resistant temperate hardwoods. Heartwood of the following 15 species were classified in perishable class; *Populus nigra* var. *italica*, *Salix sachalinensis*, *Pterocarya rhoifolia*, *Carpinus tschonoskii*, *Alnus firma* var. *hirtella*, *Ulmus lacinata*, *Magnolia kobus*, *Phellodendron amurense*, *Ailanthus altissima*, *Meliosma rigida*, *Tilia japonica*, *Idesia polycarpa*, *Acanthopanax sciadophylloides*, *Ligustrum lucidum* and *Fraxinus mandschurica* var. *serrata*. Variances of decay resistance among species and families were not much different from those for Basidiomycotina ever reported in the literature.

Twenty-four species of tropical hardwoods were designated to be very resistant against *C. globosum*. In contrast with the case of temperate hardwoods, there was a tendency that dense and/or extractive-rich species became more susceptible to decay after treatment with hot methanol. This was shown more notably in the case of *C. versicolor*. *Ochano-*

*stachys amentacea*, *Scorodocarpus borneensis*, *Eusideroxylon zwageri*, *Cantleya corniculata*, *Shorea exelliptica*, *Shorea hypoleuca* and *Shorea laevis* retained high resistance even after the treatment.

Very low ability of *C. globosum* to degrade softwoods was evidenced here with all tested species. The greater part of species retained high resistance after treatment with hot methanol. *C. versicolor* also could not cause severe weight loss of softwoods. However, extractive-rich species were more resistant against this fungus, and most of these species became less resistant after extraction with hot methanol. It can be concluded from these results that the role of extractives in softwoods with higher resistance against soft rot fungi is generally insignificant.

# CHAPTER 4 EFFECTS OF PRE-TREATMENTS OF WOOD ON THE WOOD-DECAYING CAPACITY OF CHAETOMIUM GLOBOSUM

As shown in previous chapter, very low ability of *C. globosum* to degrade softwoods was demonstrated with various wood species. Resistance of hardwoods against this fungus varied greatly with species, genera and families. Such variances of decay resistance were not so much different from those for Basidiomycotina reported in the literature. Thus, *C. globosum*, as well as white rot fungi, has been characterized by the preference for hardwoods. However, as described in Chapter 3, higher resistance of woods against white rot fungi was often depressed significantly by removal of extractives, whereas that against *C. globosum* was not so much. This has been shown more obviously in softwoods than in hardwoods.

A higher xylanase activity than the mannanase activity in culture filtrates from *C. globosum*[67], rapid growth and consumption of carbon source in xylose- and xylan media inoculated with this fungus [117] may partly explain the greater susceptibility of hardwoods to soft rot fungi. However, these evidences are still insufficient to enable us to establish a conclusive correlation between the hemicellulase activities of soft rot fungi and the hemicellulose compositions of the preferred wood, since enzyme activities for glucuronoxylan, the main hemicellulose of hardwoods, and glucomannan, the main hemicellulose of softwoods, and also amount of growth and consumption rate for xylose medium and mannose medium, were not so different.

The fact that soft rot fungi are isolated frequently from softwoods in wet- and burial conditions and are known to cause the severe destruction of cell walls of these woods[58] suggests the presence of unknown effects by which the higher resistance of softwoods against soft rot fungi might be depressed at a great extent in such conditions. In this chapter, the effects of some biological- and chemical treatments of softwoods on the wood-decaying capacity of *C. globosum* are investigated. For the purpose of further comparison, data are included on a hardwood *Fagus crenata* and on white rot- and brown rot fungi.

## 4-1 Biological treatment[120]

The main components of woody cell walls - cellulose, hemicelluloses and lignin - are degraded by various groups of organism to different extents. However, the intimate association of the lignin with the polysaccharides apparently makes wood resistant to decomposition by both enzymes and whole organisms that can decompose the isolated wood polysaccharides[71]. In the white rot, polysaccharides and lignin are attacked more or less simultaneously; in brown rot and soft rot, on the other hand the polysaccharides are principally utilized. Although lignin can be degraded by certain microorganisms, it can not be regarded as a favorable or a good energy-yielding substrate[107]. The ability of white rot fungi to decompose lignin might therefore be regarded as effective means to gain access to the polysaccharides in lignified cell wall[48,72]. Brown rot fungi, however, are able to utilize the polysaccharides in lignified

cell walls without causing any significant degradation of the lignin. Although a soft rot fungus, *C. globosum*, has been shown to attack the polysaccharides similarly in beech wood[81,105,108], untreated softwoods are virtually unattacked by any of soft rot fungi. Bailey et al.[8] proposed the existence of "pre-cellulolytic system" in organisms capable of utilizing the cellulose in wood. This term was used by them simply to describe all the steps that are necessary for the initiation of cellulose degradation. In this section, the effect of pre-exposure to certain wood-decaying fungi on the susceptibility of softwoods to *C. globosum* is investigated.

## Materials and Methods

### *Wood*

Wood blocks, 2.0 ($t$) × 2.0 ($r$) × 0.5 ($l$) (cm), were prepared from the heartwoods of two softwoods (*Pinus densiflora* Sieb. et Zucc. and *Cryptomeria japonica* D. Don) and one hardwood (*Fagus crenata* Blume). Blocks were extracted with ethanol-benzene (1:1) for 8 hours and soaked in warm water (50°C) for 4 hours before pre-exposure to fungal attack.

### *Pre-exposure of wood to fungal attack*

Fungal species used for the pre-exposure treatment are listed in Table 24. The treatment was carried out by the sand-block method, using cylindrical glass bottles described in Chapter 1. The composition of the nutrient solution for the treatment with 9 Basidiomycotina fungi is the same as that for the decay test using *C. versicolor* described in Chapter 3. The solution for the treatment with *Trichoderma viride* is

Table 24. Fungal species used in the pre-exposure treatment of wood.

| Name | Type | Strain* |
|---|---|---|
| *Coniophora puteana* Karst. | brown rot | IFO 6275 |
| *Serpula lacrymans* S. F. Gray | brown rot | IFO 8697 |
| *Tyromyces palustris* Murr. | brown rot | FES 0507 |
| *Coriolus versicolor* Quél. | white rot | FES 1030 |
| *Ganoderma lucidum* Karst. | white rot | M-1 |
| *Lenzites betulina* Fr. | white rot | FES L5b |
| *Cryptoderma pini* Imazeki | white rot | FES F15c |
| *Pycnoporus coccineus* Bond. et Sing. | white rot | FES Pslh |
| *Schizophyllum commune* Fr. | white rot | FES Sch2a |
| *Trichoderma viride* Pers. ex Fr. | mould | IFO 4847 |

*IFO: Institute for Fermentation, Osaka, Japan.

FES: Government Forest Experiment Station, Tokyo, Japan.

M-1: Morimoto Yokin-en, Kyoto, Japan.

the same as that for the test using *C. globosum*. Wood blocks for the pre-exposure to *T. viride* were soaked in sterilized distilled water after the sterilization with propylene oxide. The temperature was maintained at 20°C for *S. lacrymans* and at 28°C for other fungi throughout three different incubation periods of 2-, 4- and 8 weeks. Three blocks each in two bottles were used in each fungal- and wood species or each incubation period. The treated blocks were sterilized by fumigation with propylene oxide, cleaned of mycelium, soaked in running water for three days and then dried to constant weight in an oven at 105°C.

*Decay test using C. globosum*

The weighed blocks were sterilized again by fumigation with propylene oxide, and soaked in sterilized distilled water before exposure

to *C. globosum*. The temperature was maintained at 28°C during the 8 week-incubation period. The decayed blocks were cleaned of mycelium and then dried to constant weight in an oven at 105°C. The percent of weight loss was calculated from initial and final weights.

## Results and Discussion

Table 25 shows the weight loss in wood blocks pre-exposured to several fungi. Among the three wood species, *C. japonica* was the most resistant against fungal attack. In cases of *S. commune* and *T. viride*, wood blocks were attacked only at a small extent. For facilitating the discussion on the relation between the weight losses by the pre-exposure treatment and those by direct exposure to *C. globosum*, the wood blocks exposed to *C. globosum* were rated into four groups according to weight losses caused by the pre-exposure. The effect of pre-exposure was examined by comparing the average value of weight loss caused by *C. globosum* in each group with that in wood blocks directly exposed to this fungus. Such an effect was examined by $t$-distribution at 5, 2 and 1 % level of significance. Table 26 shows the results with the effect of pre-exposure treatment on the wood-attacking capacity of *C. globosum*. A positive (negative) effect means the case in which weight loss in treated blocks was larger (smaller) than that in untreated blocks. Unexpectedly, a positive effect was found significant only in a few combinations of fungal and wood species. Although the occurrence of positive effect was oftener in the case of *P. densiflora* than were in those of the other wood species, the extent of acceleration of attack was not

Table 25. The loss of weight in wood blocks pre-exposured to several fungi.

| Fungus | Wood | Loss of weight (%) | | |
|---|---|---|---|---|
| | | Incubation period (week) | | |
| | | 2 | 4 | 8 |
| *Coniophora puteana* | *Pinus densiflora* | 8.51 | 21.48 | 26.33 |
| | *Cryptomeria japonica* | 1.75 | 1.54 | 4.63 |
| | *Fagus crenata* | 0.29 | 3.56 | 19.56 |
| *Serpula lacrymans* | *Pinus densiflora* | 4.22 | 16.39 | 40.55 |
| | *Cryptomeria japonica* | 1.86 | 10.89 | 27.54 |
| | *Fagus crenata* | 0.57 | 1.18 | 21.71 |
| *Tyromyces palustris* | *Pinus densiflora* | 4.96 | 6.35 | 9.03 |
| | *Cryptomeria japonica* | 5.60 | 7.26 | 11.87 |
| | *Fagus crenata* | 0.56 | 2.07 | 3.28 |
| *Coriolus versicolor* | *Pinus densiflora* | 0.71 | 3.34 | 13.78 |
| | *Cryptomeria japonica* | 1.11 | 4.11 | 12.87 |
| | *Fagus crenata* | 6.48 | 17.51 | 45.52 |
| *Ganoderma lucidum* | *Pinus densiflora* | 0.66 | 1.07 | 8.06 |
| | *Cryptomeria japonica* | 2.40 | 2.68 | 2.59 |
| | *Fagus crenata* | 0.86 | 7.09 | 33.85 |
| *Lenzites betulina* | *Pinus densiflora* | 3.06 | 10.59 | 17.67 |
| | *Cryptomeria japonica* | 1.69 | 6.97 | 14.07 |
| | *Fagus crenata* | 7.08 | 21.73 | 44.57 |
| *Cryptoderma pini* | *Pinus densiflora* | 2.01 | 1.96 | 1.83 |
| | *Cryptomeria japonica* | 0.38 | 1.32 | 1.18 |
| | *Fagus crenata* | 1.03 | 2.54 | 4.40 |
| *Pycnoporus coccineus* | *Pinus densiflora* | 2.08 | 2.63 | 4.92 |
| | *Cryptomeria japonica* | 0.76 | 1.53 | 1.86 |
| | *Fagus crenata* | 5.22 | 19.50 | 41.04 |
| *Schizophyllum commune* | *Pinus densiflora* | 2.18 | 2.42 | 2.25 |
| | *Cryptomeria japonica* | 1.90 | 1.79 | 1.60 |
| | *Fagus crenata* | 0.67 | 0.73 | 1.54 |
| *Trichoderma viride* | *Pinus densiflora* | 1.45 | 1.95 | 3.18 |
| | *Cryptomeria japonica* | 2.20 | 2.49 | 2.57 |
| | *Fagus crenata* | 0.88 | 1.32 | 1.54 |

still so large even in the case. However, it is clear that so far as *P. densiflora* is concerned this wood became apparently less resistant after pre-exposure to certain white rot fungi, especially to *Ganoderma lucidum*.

White rot fungi derive nourishment from all the major constituents of the wood cell walls. However, they differ in relarive rates to remove the major components; some, such as *Polyporus berkeleyi*, remove the lignin faster than either the cellulose or the hemicelluloses, especially in early stages of decay[66]. Others, such as *Coriolus versicolor*, remove the three major components approximately simultaneously[66]. Apparently only a few fungi remove the carbohydrates somewhat more rapidly than the lignin[66]. On the other hand, brown rot fungi mainly decompose the polysaccharides in wood and usually cause only a small loss in the lignin. The cellulose and hemicelluloses are removed at about the same relative rates by various species of brown rot fungi[33,109].

A positive effect may be due to; (1) removal of some hindrances which are present in unknown associations of the three major components to enzyme activity of *C. globosum*, (2) depolymerization and structural modification of these components, (3) supplement of nitrogen source derived from dead fungal cells, and (4) facilitation of penetration of *C. globosum* into cell lumen via bore hole previously produced by test fungus. A negative effect may be attributed to; (1) insufficient removal of toxic materials produced by test fungus, (2) exhaustion of food reserves in parenchyma cells, and (3) depletion of polysaccharides available to *C. globosum*.

Table 26. The effect of pre-exposure to several fungi on the wood-attacking capacity of *Chaetomium globosum*.

| Fungus \ Wood | *Pinus densiflora* | | | *Cryptomeria japonica* | | | *Fagus crenata* | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C |
| *Coniophora puteana* | 6.90 | 3.72 | ++ | 0.30 | 2.83 | | 0.46 | 37.03 | |
| | 14.90 | 3.01 | | 1.80 | 3.07 | ++ | 4.86 | 45.08 | + |
| | 24.24 | 2.45 | - | 3.56 | 1.88 | | 17.48 | 32.74 | |
| | 32.98 | 2.31 | | 5.49 | 2.24 | | 23.75 | 30.47 | |
| *Serpula lacrymans* | 4.22 | 3.15 | | 1.86 | 1.09 | --- | 0.57 | 44.08 | |
| | 16.90 | 2.98 | | 7.87 | 1.99 | | 1.18 | 57.30 | +++ |
| | 35.75 | 3.85 | +++ | 15.14 | 2.23 | | 14.34 | 39.05 | |
| | 50.16 | 2.52 | | 31.80 | 1.84 | | 25.39 | 39.05 | |
| *Tyromyces palustris* | 1.10 | 1.74 | -- | 2.62 | 1.49 | | 0.43 | 45.42 | +++ |
| | 5.09 | 1.70 | --- | 6.19 | 1.50 | | 1.34 | 40.63 | |
| | 7.55 | 2.38 | | 9.36 | 1.85 | | 2.68 | 39.10 | |
| | 9.03 | 3.02 | | 12.56 | 1.71 | | 7.05 | 37.51 | |
| *Coriolus versicolor* | 0.71 | 3.39 | | 1.11 | 1.59 | - | 3.94 | 35.39 | |
| | 3.34 | 6.15 | +++ | 4.11 | 2.51 | + | 11.83 | 36.43 | |
| | 12.88 | 2.71 | | 12.01 | 0.99 | --- | 32.56 | 33.23 | |
| | 15.88 | 4.97 | +++ | 14.61 | 0.24 | --- | 50.73 | 24.07 | |
| *Ganoderma lucidum* | 0.27 | 3.67 | ++ | 0.57 | 1.48 | - | 0.73 | 32.09 | - |
| | 1.68 | 3.62 | +++ | 2.04 | 2.25 | | 6.35 | 26.93 | --- |
| | 4.36 | 4.10 | | 2.88 | 1.70 | | 23.49 | 36.43 | |
| | 12.05 | 7.46 | +++ | 5.11 | 1.62 | | 37.45 | 28.22 | -- |
| *Lenzites betulina* | 1.17 | 1.44 | --- | 1.86 | 2.04 | | 7.08 | 28.00 | -- |
| | 6.42 | 3.85 | | 6.81 | 1.81 | | 20.15 | 33.38 | |
| | 17.09 | 5.73 | +++ | 10.52 | 2.12 | | 38.92 | 20.92 | --- |
| | 27.52 | 5.22 | | 16.73 | 2.23 | | 51.25 | 24.75 | --- |

Table 26. The effect of pre-exposure to several fungi on the wood-attacking capacity of *Chaetomium globosum* (Continued).

| Fungus \ Wood | *Pinus densiflora* | | | *Cryptomeria japonica* | | | *Fagus crenata* | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C |
| *Cryptoderma pini* | 0.73 | 1.60 | --- | 0.22 | 1.45 | | 0.90 | 46.18 | ++ |
| | 1.95 | 1.07 | --- | 0.54 | 1.05 | -- | 2.23 | 36.63 | |
| | 2.46 | 1.86 | | 1.13 | 1.37 | --- | 3.39 | 34.17 | |
| | 5.74 | 3.75 | | 1.54 | 0.56 | --- | 5.84 | 24.56 | --- |
| *Pycnoporus coccineus* | 1.93 | 1.88 | --- | 0.64 | 2.01 | | 5.22 | 26.13 | - |
| | 2.87 | 1.07 | --- | 1.07 | 1.78 | | 16.37 | 41.67 | |
| | 5.28 | 4.17 | | 1.65 | 0.60 | --- | 25.24 | 31.50 | |
| | 7.77 | 8.59 | +++ | 2.05 | 0.18 | --- | 43.55 | 36.12 | |
| *Shizophyllum commune* | 1.73 | 1.16 | --- | 0.48 | 1.30 | | 0.43 | 34.81 | |
| | 2.23 | 1.36 | --- | 1.56 | 0.81 | --- | 0.76 | 42.33 | |
| | 2.45 | 1.23 | --- | 2.17 | 1.11 | -- | 1.24 | 38.70 | |
| | 2.61 | 0.92 | --- | 2.75 | 1.49 | | 1.69 | 45.31 | +++ |
| *Trichoderma viride* | 1.39 | 1.22 | --- | 2.07 | 1.01 | - | 0.84 | 42.84 | |
| | 1.92 | 0.92 | --- | 2.27 | 1.60 | | 1.18 | 46.36 | |
| | 3.00 | 0.95 | --- | 2.55 | 1.16 | -- | 1.37 | 48.68 | +++ |
| | 3.54 | 0.67 | --- | 3.16 | 0.95 | --- | 1.76 | 45.06 | |
| None | — | 2.86 | — | — | 2.09 | — | — | 37.28 | — |

A: Percent of weight loss by pre-exposure treatment.

B: Percent of weight loss by exposure to *C. globosum*.

C: Effect of pre-exposure treatment.

+, ++ and +++ mean positive effect significant at 5, 2 and 1 % level, respectively.

-, -- and --- mean negative effect significant at 5, 2 and 1 % level, respectively.

All values are expressed on the basis of the weight of extractive-free wood before pre-exposure treatment.

Chemical analysis and microscopic observation of pre-exposed wood were not carried out in the present experiment. It is therefore not clear which causal agent is main among the effects in each combination of fungus and wood. However, it can be seen from these results that lower attacking capacity of *C. globosum* to softwoods is not accelerated so much by biological treatment, even by the action of white rot fungi which cause to degrade and modify the lignin and its association with polysaccharides.

## 4-2 Chemical treatment

### (1) Alkali- and acid treatments[121)]

Cold soda treatments were found to give little effect on the lignin and pentosan content of pulps[132)]. Bland and Watson[10)] demonstrated that most of the lignin was retained in cold soda semichemical pulps and that its properties showed no difference from those of original lignin. Cold soda treatments therefore would be expected to cause some effect on the lignin with little delignification. Bland and Menshun[11)] investigated the effect of alkali pre-treatment of the wood on the yield and carbohydrate retention of the lignin by extraction with acetone of the treated milled wood. They found that a mild alkali pre-treatment resulted in a large yield of lignin and suggested that this pre-treatment appeared to hydrolyse the bonds between lignin and carbohydrates. They later concluded[12)] that a large part of the lignin was bound to polysaccharides by alkali resistant bonds and these could be ether bonds as

deduced by Brownell[16]. On the other hand, from the results with treatments of lignin-carbohydrate complexes with alkali and acid, Bolker and Wang[15] concluded that mild treatment with acid was effective in separating the lignin from the carbohydrate while alkali treatment was not. They considered that these results support previous proposals [13,14,47,57,84] that lignin and carbohydrate in plants may be joined by acid-labile bonds, or that the breaking of such bonds is at least necessary before effective separation of the two materials.

In the first part of this section, the effect of mild alkali- and acid treatments on the susceptibility of softwoods to *C. globosum* is investigated.

## Materials and Methods

### *Wood*

Size of wood blocks and wood species used in the experiment are the same as described in 4-1. Blocks were extracted with ethanol-benzene (1:1) for 8 hours and soaked in warm water (50°C) for 4 hours before alkali- and acid treatments.

### *Alkali- and acid treatments of wood*

Wood blocks were soaked in various concentrations of sodium hydroxide (30 ml/g wood) or hydrochloric acid (30 ml/g wood) for 20 hours at 20°C, and subsequently well washed with water, air dried and then dried again in an oven at 105°C. Concentrations of sodium hydroxide are as follows:

for the treatment of *P. densiflora* and *F. crenata*

0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 *M*,

for the treatment of *C. japonica*

0.1, 0.2, 0.4, 1.0, 2.0 and 4.0 *M*.

Concentrations of hydrochloric acid are: 0.1, 0.2, 0.4, 1.0, 2.0 and 4.0 for all wood species.

*Decay test*

Decay test was made by the same procedures described in 4-1.

*Determination of lignin in wood*

To define the relation between the weight losses and the decrease in lignin amounts by treatments, wood shavings (0.3 mm thick in longitudinal direction, cut to about 4 mm in the fibre direction) were prepared from the same wood specimens and treated with alkali or acid under the same condition as mentioned above. Concentrations of sodium hydroxide are: 0.1, 0.5 and 2.0 *M*, and those of hydrochloric acid are: 0.2, 1.0 and 4.0 *M*. The Klason lignin content in treated wood shavings was determined by the JIS P 8008-1961.

## Results and Discussion

Table 27 shows the loss of weight by alkali- and acid treatments and the Klason lignin content in treated wood shavings. Each value is an average of data from three separate samples. From the difference between the loss of weight and the decrease in the Klason lignin content during the treatment, the decrease in materials other than Klason lignin was roughly estimated as follows:

$$\text{Loss of materials other than Klason ligin in \%} = \frac{L - (K_0 - K_1)}{100 - K_0} \times 100$$

where $L$ is the percent of weight loss in wood by treatment, $K_0$ is the percent of the Klason lignin content in untreated wood, and $K_1$ is the percent of the Klason lignin content in treated wood. As shown in Table 27, both treatments caused a certain extent of delignification but they are not selective in removing lignin only from all of the three wood species. *C. japonica* was more resistant against the alkali treatment than others. In the case of *F. crenata*, lignin and other substances were removed at about the same relative rates by the alkali treatment. In the cases of two softwoods, lignin was removed more rapidly than were other substances. It might possibly be hemicelluloses that was removed together with the lignin by alkali treatment.

The wood blocks exposed to *C. globosum* were rated into four groups according to weight losses caused by alkali or acid treatment. The effect of treatment was examined by comparing the average value of weight loss caused by *C. globosum* in each group with that in untreated blocks, and the data was examined by $t$-distribution at 5, 2 and 1 % level of significance. Table 28 shows the results with the effect of alkali- and acid treatments on the wood-aatacking capacity of *C. globosum*. Positive- and negative effects were found significant only in a few cases. However, the occurrence of positive effect was oftener in the case of *P. densiflora*. This is similar to the case of the wood subjected to the pre-exposure treatment described in 4-1.

Table 27. The loss of weight by alkali- and acid treatments and the Klason lignin content in treated wood shavings.

| Treatment \ Wood | M | *Pinus densiflora* | | | | *Cryptomeria japonica* | | | | *Fagus crenata* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | A | B | C | D | A | B | C | D |
| Sodium hydroxide | 0.1 | 5.2 | 26.8 | 6.3 | 4.8 | 4.7 | 33.1 | 4.5 | 4.6 | 5.2 | 25.1 | 6.0 | 4.9 |
| | 0.5 | 7.5 | 26.1 | 8.7 | 7.0 | 5.0 | 32.4 | 6.9 | 4.0 | 9.8 | 24.1 | 9.7 | 9.8 |
| | 2.0 | 8.9 | 23.7 | 17.1 | 5.6 | 6.0 | 31.4 | 9.8 | 4.0 | 12.1 | 23.9 | 10.5 | 12.7 |
| Hydro-chloric acid | 0.2 | 2.4 | 27.4 | 4.2 | 1.7 | 2.7 | 34.0 | 2.3 | 2.9 | 1.2 | 26.2 | 1.9 | 1.0 |
| | 1.0 | 2.8 | 27.4 | 4.2 | 2.2 | 2.9 | 33.8 | 2.9 | 2.9 | 1.6 | 26.2 | 1.9 | 1.5 |
| | 4.0 | 4.1 | 27.0 | 5.6 | 3.5 | 3.7 | 33.7 | 3.2 | 4.0 | 2.8 | 25.8 | 3.4 | 2.6 |
| None | — | 0 | 28.6 | 0 | 0 | 0 | 34.8 | 0 | 0 | 0 | 26.7 | 0 | 0 |

M: Concentration (*M*).

A: Percent of weight loss by treatment.

B: Klason lignin content.

C: Percent of loss of Klason lignin.

D: Percent of loss of matérials other than Klason lignin.

Table 28. The effect of alkali- and acid treatments of wood on the wood-attacking capacity of *Chaetomium globosum*.

| Treatment \ Wood | *Pinus densiflora* | | | *Cryptomeria japonica* | | | *Fagus crenata* | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | A | B | C | A | B | C |
| Sodium hydroxide | 2.94 | 1.51 | | 2.76 | 0.42 | - | 2.14 | 44.34 | |
| | 5.70 | 3.49 | | 3.21 | 1.08 | | 4.27 | 56.52 | +++ |
| | 8.47 | 4.78 | + | 3.52 | 1.72 | | 6.89 | 44.21 | |
| | 10.67 | 3.17 | + | 3.80 | 1.89 | | 8.09 | 38.76 | |
| Hydro-chloric acid | 1.67 | 4.70 | +++ | 1.32 | 1.06 | | 1.01 | 26.24 | --- |
| | 1.98 | 4.67 | +++ | 1.57 | 1.03 | | 1.33 | 30.96 | |
| | 2.58 | 4.12 | +++ | 2.09 | 0.71 | | 1.74 | 30.09 | |
| | 3.49 | 3.00 | | 3.21 | 0.74 | | 2.41 | 35.36 | |
| None | 0 | 1.82 | — | 0 | 1.06 | — | 0 | 37.28 | — |

A: Percent of weight loss by treatment.

B: Percent of weight loss by exposure to *C. globosum*.

C: Effect of treatment.

+ and +++ mean positive effect significant at 5 and 1 % level, respectively.

- and --- mean negative effect significant at 5 and 1 % level, respectively.

All values are expressed on the basis of the weight of extractive-free wood before treatment.

As shown in Table 27, delignifying effect was larger in the alkali treatment than was in the acid treatment. However, in cases of the two softwoods, the extent of acceleration of decay by *C. globosum* was not different between the two treatments. In the case of *P. densiflora*, small extent of the acceleartion was found in both treatments, whereas it was not in the case of *C. japonica*. Both treatments apparently caused not only the delignification of wood but also some extent of modification of lignin itself or its association with the polysaccharides. Although it is not clear at this stage which agent is main in the acceleration of decay, mild treatment with alkali or acid is not sufficient to make softwoods less resistant as well as beech wood.

(2) Partial delignification with acidified sodium chlorite[122)]

As mentioned above, all of the four treatments - removal of extractives , pre-exposure to fungal attack, alkali treatment and acid treatment - were not effective substantially for the acceleration of attack on softwoods by *C. globosum*. The greater resistance of softwoods to soft rot attack has often been attributed to a physical blocking of the enzymes by the higher lignin content[103,105)]. Brown rot fungi, however, are able to utilize the carbohydrates of softwood cell walls without causing any significant depletion of the lignin. Significance of the higher lignin content therefore seems to vary with the type of decay. Effect of delignification should be investigated on the susceptibility of softwoods to wood-decaying fungi. Bailey et al.[8)] reported that chlorite treatment of *Picea abies* bringing about 2 % of weight loss made

this wood nearly as susceptible as beech to three species of soft rot fungi, but that such a treatment did not influence on the susceptibility of this wood to brown rot fungi.

Many physicochemical studies have been made on the important commercial pulping processes. Ahlgren and Goring[2] found that the chlorite process was selective in removing lignin from spruce wood (*Picea mariana*) during the first 60 % of delignification, and concluded, by comparing that with earlier data[90,111,137,138], that the selectivity of the chlorite procedure was much greater than conventional pulping processes.

In the second part of this section, the effect of the chlorite delignification of softwoods on the wood-decaying capacity of *C. globosum* is studied at various stages of delignification. For comparison, the study covered a hardwood (*Fagus crenata*) and some white rot- and brown rot fungi.

## Materials and Methods

### *Wood*

The names of 44 softwood- and 1 hardwood timber species employed in the experiment are listed in Table 29. Sampling of wood specimen was made from the intermediate portion of heartwood, because of the well established trend of increasing decay resistance from the innermost to the outermost heartwood. Wood specimens were used in two forms; (1) 2.0 ($t$) × 2.0 ($r$) × 0.5 ($l$) (cm) blocks, and (2) 0.3 mm thick longitudinal shavings cut to about 4 mm in the fibre direction. Wood blocks were prepared from all of the species and subjected to the decay test.

Table 29. Timber species used in the partial chlorite delignification and the decay test.

| No. | Botanical name | Common name | |
|---|---|---|---|
| | | Japanese | English |
| 1 | *Ginkgo biloba* L. | Icho | Maidenhair tree |
| 2 | *Taxus cuspidata* Sieb. et Zucc. | Ichii | Japanese yew |
| 3 | *Torreya nucifera* Sieb. et Zucc. | Kaya | Japanese Torreya |
| 4 | *Podocarpus macrophylla* D. Don | Inumaki | Longleaf Podocarp |
| 5 | *Podocarpus nagi* Zoll. et Moritz. | Nagi | Nagi Podocarp |
| 6 | *Cephalotaxus harringtonia* K. Koch | Inugaya | Japanese plum yew |
| 7 | *Abies firma* Sieb. et Zucc. | Momi | Japanese fir |
| 8 | *Abies mariesii* Mast. | Aomoritodomatsu | Maries' fir |
| 9 | *Abies sachalinensis* Fr. Schm. | Todomatsu | Sachalin fir |
| 10 | *Abies sachalinensis* Fr. Schm. var. *mayriana* Miyabe et Kudo | Aotodomatsu | (Fir, Spruce) |
| 11 | *Pseudotsuga japonica* Beissn. | Togasawara | Japanese Douglas fir |
| 12 | *Tsuga sieboldii* Carr. | Tsuga | Japanese hemlock |
| 13 | *Picea glehnii* Mast. | Akaezomatsu | Glehn's spruce |
| 14 | *Picea jezoensis* Carr. | Ezomatsu | Yezo spruce |
| 15 | *Picea abies* Karst. | Doitsutohi | Spruce |
| 16 | *Larix leptolepsis* Gord. | Karamatsu | Japanese larch |
| 17 | *Larix gmelini* Ledeb. | Guimatsu | Kurile larch |
| 18 | *Keteleeria davidiana* Beissn. | Yusan | Aburasugi |
| 19 | *Pinus densiflora* Sieb. et Zucc. | Akamatsu | Japanese red pine |
| 20 | *Pinus pentaphylla* Mayr. | Himekomatsu | Japanese white pine |
| 21 | *Pinus thunbergii* Parl. | Kuromatsu | Japanese black pine |
| 22 | *Pinus tabulaeformis* Carr. | Manshukuromatsu | Manchurian pine |
| 23 | *Pinus radiata* D. Don | Rajiatamatsu | Montrey pine |
| 24 | *Pinus rigida* Mill. | Rigidamatsu | Black pine |
| 25 | *Pinus taeda* L. | Tedamatsu | Torchpine |

Table 29. Timber species used in the partial chlorite delignification and the decay test (Continued).

| No. | Botanical name | Common name | |
|---|---|---|---|
| | | Japanese | English |
| 26 | *Pinus sylvestris* L. | Oshuakamatsu | Scots pine |
| 27 | *Pinus nigra* Arn. | Oshukuromatsu | Corcican black pine |
| 28 | *Pinus strobus* L. | Sutorobumatsu | White pine |
| 29 | *Pinus virginiana* Mill. | Bajiniamatsu | Virginia pine |
| 30 | *Pinus elliottii* Engelm. | Eriottimatsu | American pitch pine |
| 31 | *Sciadopitys verticillata* Sieb. et Zucc. | Koyamaki | Umbrella pine |
| 32 | *Sequoia sempervirens* Endl. | Sekoia | Redwood |
| 33 | *Metasequoia glyptostroboides* Hu et Cheng | Metasekoia | Dawn redwood |
| 34 | *Glyptostrobus pensilis* K. Koch | Suisho | Swamp cypress |
| 35 | *Taxodium distichum* Rich | Numasugi | Bald cypress |
| 36 | *Cryptomeria japonica* D. Don | Sugi | Japanese Cryptomeria |
| 37 | *Cunninghamia konisii* Hayata | Randaisugi | Formosa fir |
| 38 | *Taiwania cryptomerioides* Hayata | Taiwansugi | Taiwania |
| 39 | *Chamaecyparis obtusa* Endl. | Hinoki | Japanese cypress |
| 40 | *Chamaecyparis pisifera* Endl. | Sawara | Sawara cypress |
| 41 | *Chamaecyparis formosensis* Matsum. | Benihi | Formosan cypress |
| 42 | *Thuja standishii* Carr. | Nezuko | Japanese arbor-vitae |
| 43 | *Thujopsis dolabrata* Sieb. et Zucc. | Asunaro | Hiba arbor-vitae |
| 44 | *Juniperus virginiana* L. | Enpitsu-byakushin | Eastern red cedar |
| 45 | *Fagus crenata* Blume | Buna | Japanese beech |

Shavings were used for the lignin determination and taken from the three species only (Nos. 19, 36 and 45, see Table 29).

*Partial delignification*

The partial delignification of the wood specimens was carried out with sodium chlorite and acetic acid. The specimens were extracted with ethanol-benzene (1:2) for 24 hours and soaked in warm water (50°C) for 4 hours before chlorite treatment.

Wood blocks:

The specimens, separately bound to the teflon plate with fine teflon strings, were placed in a beaker containing sodium chlorite (70 g $NaClO_2$/ 30 g wood/litre solution) and acetic acid (30 ml). The solutions were maintained at 40°C and stirred slowly throughout the treatment by a magnetic stirrer. Six blocks each from all tested wood species were treated for 6 hours. On the three wood species (Nos. 19, 36 and 45), in addition, sets of 6 blocks each of all wood species were treated for different lengths of time, 24 hours at the longest.

Wood shavings:

The specimens were placed in a beaker containing the solution with the same amount of chemicals as mentioned above. Batches of 1 g of wood were treated for different lengths of time under the same condition. After the chlorite treatment, the wood specimens were rinsed in running water, and air-dried before drying to constant weight in an oven at 105°C

*Lignin determination*

The Klason lignin content was determined by the JIS P 8008-1961. The acid-soluble lignin content was determined on the hydrolysate from

the Klason lignin by measuring ultraviolet absorption at 205 nm in 1 cm quartz cells by a Shimadzu MPS-50 spectrophotometer. Fulfural and hydroxymethyl fulfural are formed on the acid treatment of carbohydrates under the conditions of hydrolysis used. However, these aldehydes give a sharp absorption maximum at about 280 nm, but relatively low absorbance at shorter wavelengths (205-210 nm). Several investigators have reported that measurement of absorbance at a wavelength in the region of 200-210 nm provides a reasonable measure of soluble lignin in wood. Usually, the amount of soluble lignin is determined as compared with some standard lignin preparations. However, some preparations, such as modified or degraded products derived from the original lignin during chlorite treatment, may have an unknown and fluctuating absorptivity. Hence, accepting that determination of soluble lignin in chlorited preparations by ultraviolet absorbance is considered as approximations, the absorptivity used is 110 $g^{-1}$ 1 $cm^{-1}$ was used for all batches of the three species according to Musha and Goring[94] and Swan[110]. The acid-soluble lignin content was calculated as follows[20]:

$$\text{Acid-soluble lignin in \%} = \frac{(A_s - A_b) \times V}{110 \times W} \times 100$$

where $A_s$ is the absorbance of the sample, $A_b$ is the absorbance of the blank, $W$ is the weight of the sample in g, and $V$ is the volume in litres of the solution.

*Test fungi*

*C. globosum* was mostly used as a test fungus. For comparison, two

species of white rot fungi, *Coriolus versicolor* Quél. (FES 1030) and *Pycnoporus coccineus* Bond. et Sing. (FES Pslh), and two species of brown rot fungi, *Coniophora puteana* Karst. (IFO 6275) and *Serpula lacrymans* S. F. Gray (IFO 8697), were occasionally used.

*Decay test*

The decay test was carried out by the sand-block method according to the procedures described in 4-1. In the decay test using *S. lacrymans* the temperature was maintained at 20°C during a 8-week incubation, in consideration of the optimum temperature for growth of this fungus.

## Results and Discussion

Table 30 shows the results obtained for the weight losses after the chlorite treatment and the exposure to *C. globosum*. The weight losses after the chlorite treatment considerably varied with species. The lowest weight loss was 1.58 % for *Chamaecyparis obtusa* (No. 39) and the highest was 21.92 % for *Larix gmelini* (No. 17). The average value of 44 softwoods was about 8 %. The resistance of softwoods to acidified sodium chlorite was classified into four classes:

I; weight loss was less than 4 % for 4 species,

II; weight loss was 4-8 % for 19 species,

III; weight loss was 8-12 % for 16 species,

IV; weight loss over 12 % for 5 species.

*Fagus crenata*, only one hardwood used in the experiment, could be classified into class III.

In the present chlorite treatment of the samples, neither adjust-

ment of pH of the solutions nor addition of chemicals was made. Therefore, it is not clear that different resistance to chemical attack observed here implies different accessibility of the reagent to lignin and polysaccharides through the cell wall of softwoods, or different rate of reaction in which several factors, such as pH, temperature and charge of chemicals in acidified chlorite solution, are concerned. However, noteworthy is the close relation between the weight losses by the treatment and those by decay. Fig. 41 shows the acceleration of decay which is given by the difference in weight losses between treated and untreated samples, as shown in Table 30. The highest acceleration was 54.06 % for *Picea glehnii* (No. 13) and the lowest was 5.93 % for *C. obtusa* (No. 39). *L. gmelini* (No. 17) exceptionally showed lower acceleration in spite of the lowest resistance to chemical attack. *F. crenata* (No. 45) was almost the same as *L. gmelini* in respect of its lower acceleration. Both woods, as shown in Table 30 and Chapter 3, have relatively lower natural decay resistance. In the case of such species, delignification treatment may be less effective for *C. globosum*.

Fig. 42 shows the summarized data for the acceleration of wood decay by *C. globosum* after partial delignification. Points on Fig. 42 show average values for four classes of resistance to chemical attack described above. *F. crenata* was excluded from the calculation. Acceleration is nearly straight within the range of weight loss by chlorite treatment from 0 to about 10 %, and may reach a maximum level at about 16 % or more.

Figs. 43-45 show the reults for the acid-soluble lignin of wood shavings from three species of wood (Nos. 19, 36 and 45) at various

Table 30. Decay resistance of 45 timber species against *Chaetomium globosum* after partial chlorite delignification (6 hours at 40°C)

| No.* | Weight loss by chl. treatment (%)** | Weight loss by decay | |
|---|---|---|---|
| | | Delignified (%)*** | Non-treated (%)**** |
| 1 | 5.34 | 19.44 | 0.68 |
| 2 | 11.72 | 35.17 | 0.51 |
| 3 | 3.63 | 12.56 | 1.94 |
| 4 | 6.44 | 16.83 | 0.00 |
| 5 | 7.37 | 23.70 | 1.10 |
| 6 | 2.10 | 21.34 | 0.68 |
| 7 | 4.24 | 10.16 | 0.94 |
| 8 | 9.30 | 33.39 | 4.37 |
| 9 | 5.17 | 34.21 | 0.59 |
| 10 | 8.04 | 38.09 | 2.61 |
| 11 | 4.86 | 22.84 | 1.46 |
| 12 | 9.94 | 27.92 | 2.87 |
| 13 | 11.57 | 58.18 | 4.12 |
| 14 | 5.48 | 41.38 | 10.95 |
| 15 | 8.06 | 43.04 | 2.83 |
| 16 | 10.08 | 38.66 | 0.82 |
| 17 | 21.92 | 27.24 | 10.54 |
| 18 | 4.57 | 16.39 | 0.19 |
| 19 | 5.26 | 30.83 | 0.00 |
| 20 | 10.38 | 31.70 | 1.64 |
| 21 | 5.27 | 29.84 | 2.02 |
| 22 | 4.62 | 23.65 | 2.27 |
| 23 | 10.32 | 44.30 | 7.30 |
| 24 | 4.81 | 28.24 | 9.00 |
| 25 | 9.92 | 56.19 | 2.37 |
| 26 | 3.38 | 13.42 | 1.22 |
| 27 | 13.89 | 43.29 | 10.20 |

Table 30. Decay resistance of 45 timber species against *Chaetomium globosum* after partial chlorite delignification (6 hours at 40°C) (Continued).

| No.* | Weight loss by chl. treatment (%)** | Weight loss by decay | |
|---|---|---|---|
| | | Delignified (%)*** | Non-treated (%)**** |
| 28 | 7.81 | 48.93 | 7.34 |
| 29 | 10.13 | 34.27 | 7.94 |
| 30 | 4.67 | 24.21 | 7.39 |
| 31 | 7.47 | 28.94 | 2.41 |
| 32 | 16.82 | 43.90 | 0.06 |
| 33 | 13.84 | 29.10 | 0.03 |
| 34 | 8.08 | 25.73 | 0.21 |
| 35 | 6.04 | 30.60 | 4.92 |
| 36 | 6.08 | 19.86 | 0.00 |
| 37 | 8.53 | 25.28 | 1.24 |
| 38 | 8.37 | 26.82 | 1.84 |
| 39 | 1.58 | 6.24 | 0.31 |
| 40 | 9.98 | 49.29 | 0.87 |
| 41 | 9.62 | 29.42 | 1.59 |
| 42 | 18.47 | 55.04 | 3.14 |
| 43 | 7.80 | 31.11 | 0.00 |
| 44 | 6.18 | 28.69 | 0.00 |
| 45 | 10.93 | 40.27 | 36.63 |

* See Table 29.

** $\frac{W_2 - W_3}{W_1} \times 100$, *** $\frac{W_3 - W_4}{W_1} \times 100$, **** $\frac{W_1 - W_4}{W_1} \times 100$.

$W_1$: Weight of original wood, $W_2$: Weight of wood extracted with ethanol-benzene, $W_3$: Weight of chlorite treated wood, $W_4$: Weight of decayed wood.

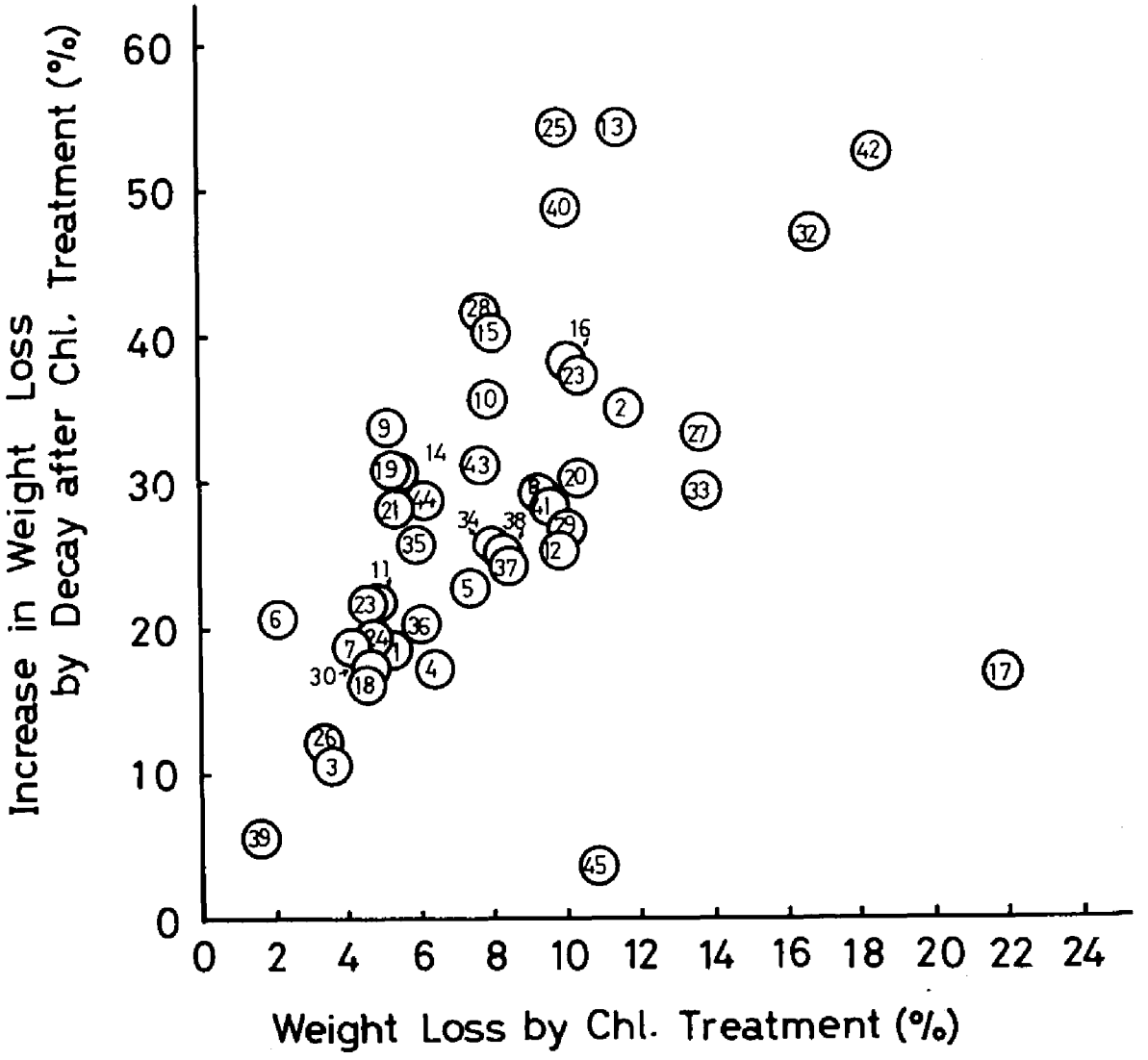



Fig. 41. Acceleration of wood-attacking capacity of *Chaetomium globosum* on 44 softwood timber species after partial chlorite delignification for 6 hours at 40°C. The points can be identified by the number given in Table 29.

delignification stages by treatment with acidified sodium chlorite. The point, shown as "not treated" on each figure, is an average of data from

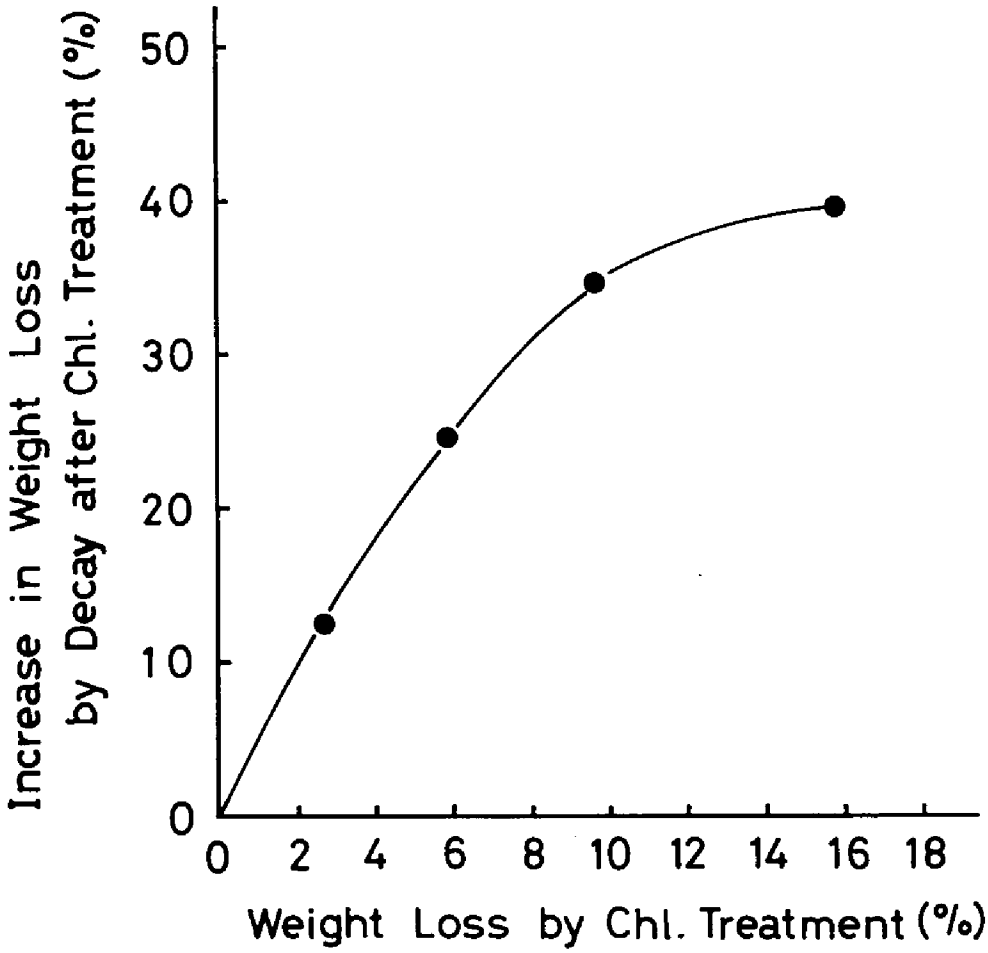



Fig. 42. Summarized data for the acceleration of wood-attacking capacity of *Chaetomium globosum* on softwoods after partial chlorite delignification for 6 hours at 40°C.

three separate Klason hydrolysates. There are several indications that the amount of acid-soluble lignin from untreated softwoods is very small. In fact, the acid-soluble lignin was only 0.26 % for *Pinus densiflora* and 0.37 % for *Cryptomeria japonica*. *F. crenata* showed relatively higher content (2.14 %). This value was lower than that of American beech

(*Fagus grandifolia*) obtained by Musha and Goring[94] but was within the range from 1 to 4 %, as reported by Schöning and Johansson[106] for hardwoods and straws.

A high level of the soluble lignin during the chlorite process agreed well with some previous reports[2,17,23]. The maximum was reached at about 9 % of Klason lignin content for *P. densiflora*, at about 14 % for *C. japonica*, and at about 10 % for *F. crenata*.

Browning[20] pointed out that a considerable portion of the total lignin is soluble after the acid treatment and the amount of insoluble residue is not a realistic measure of lignin content. According to him, the total lignin content was determined as insoluble Klason lignin plus the ultraviolet-estimated acid-soluble lignin.

The pattern of lignin removal from the three species of wood during the chlorite treatment has been shown in Figs. 46-48. Difference between the losses in Klason and total lignins is evident for each species. For facilitating the discussion, smoothed values from the curves in Figs. 46-48 are compiled in Table 32. In addition, the estimated lignin losses which were calculated on the assumption that the chlorite procedure is selective in removing lignin only are shown in this table. In the case of *C. japonica*, the smoothed values nearly agreed with the estimated values throughout the process. On the other hand, in the cases of *P. densiflora* and *F. crenata*, the two values did not agree during the whole stage. Such disagreement was more conspicuous for *F. crenata*. In both woods, considering from the material balances, some substances other than lignin must be removed during the process. Therefore, the

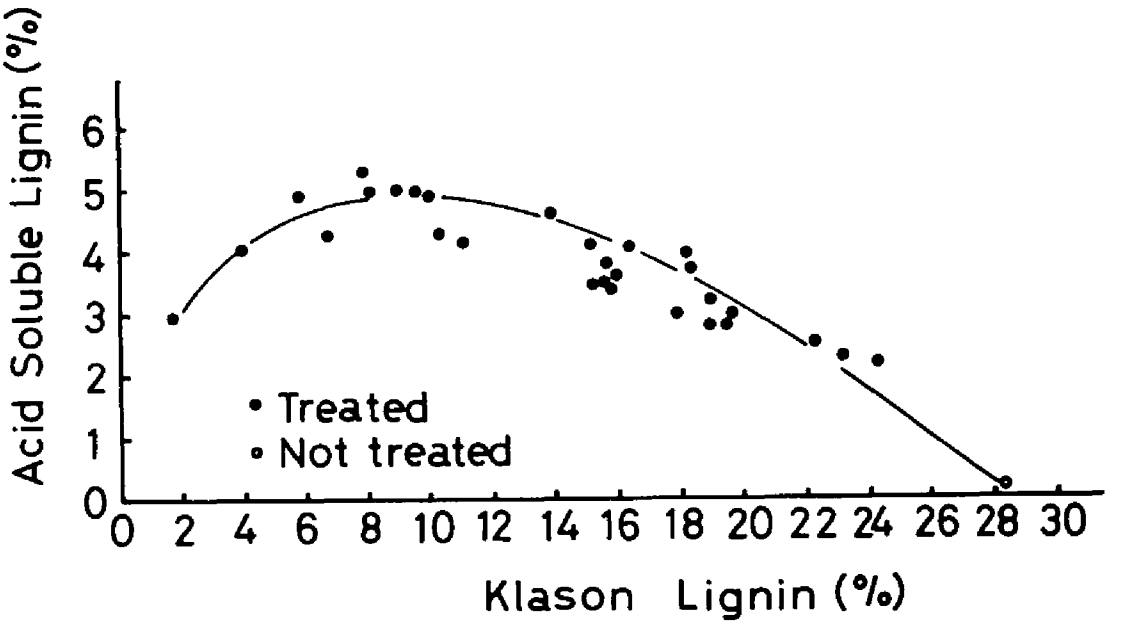



Fig. 43. The amount of acid-soluble lignin from chlorite treated wood of *Pinus densiflora* at different stages of delignification.

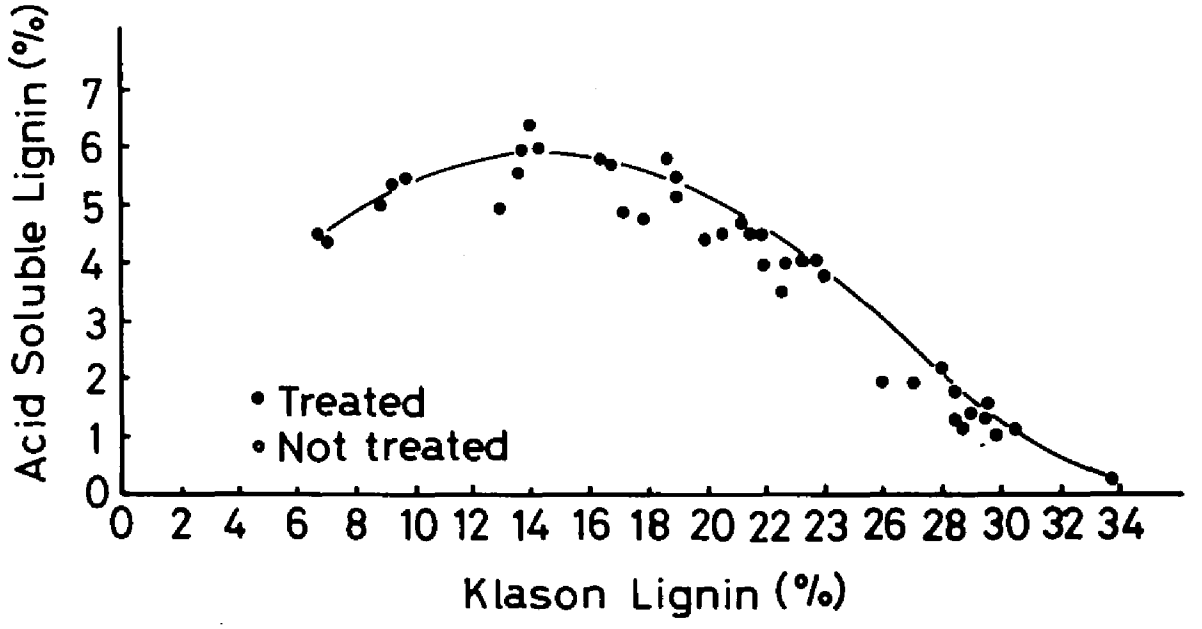



Fig. 44. The amount of acid-soluble lignin from chlorite treated wood of *Cryptomeria japonica* at different stages of delignification.

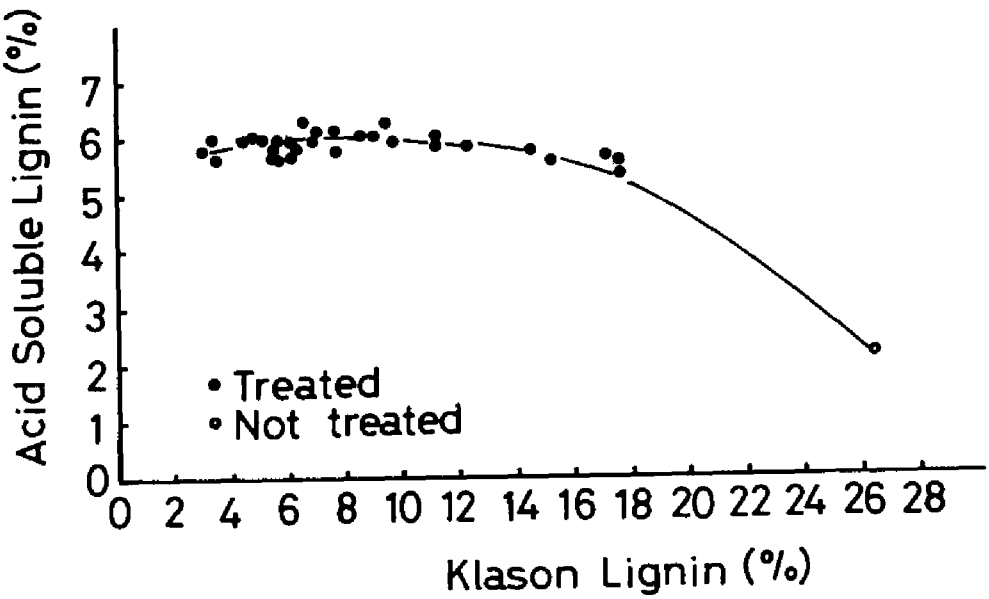



Fig. 45. The amount of acid-soluble lignin from chlorite treated wood of *Fagus crenata* at different stages of delignification.

chlorite process proved to be selective in removing lignin only from *C. japonica* as was from *Picea mariana*. However, it can be considered that the process was less selective in the cases of *P. densiflora* and *F. crenata*.

The decayed blocks were rated into eight groups according to weight losses caused by chlorite treatment before decay. Average values of weight losses after treatment and exposure to fungal attack were calculated for each group. Figs. 49-53 show the results with the average weight losses in chlorite treated wood for each group after exposure to fungal attack. In the case of *P. densiflora* attacked by *C. globosum* (Fig. 49), the acceleration curve was steep during the first 4 % of

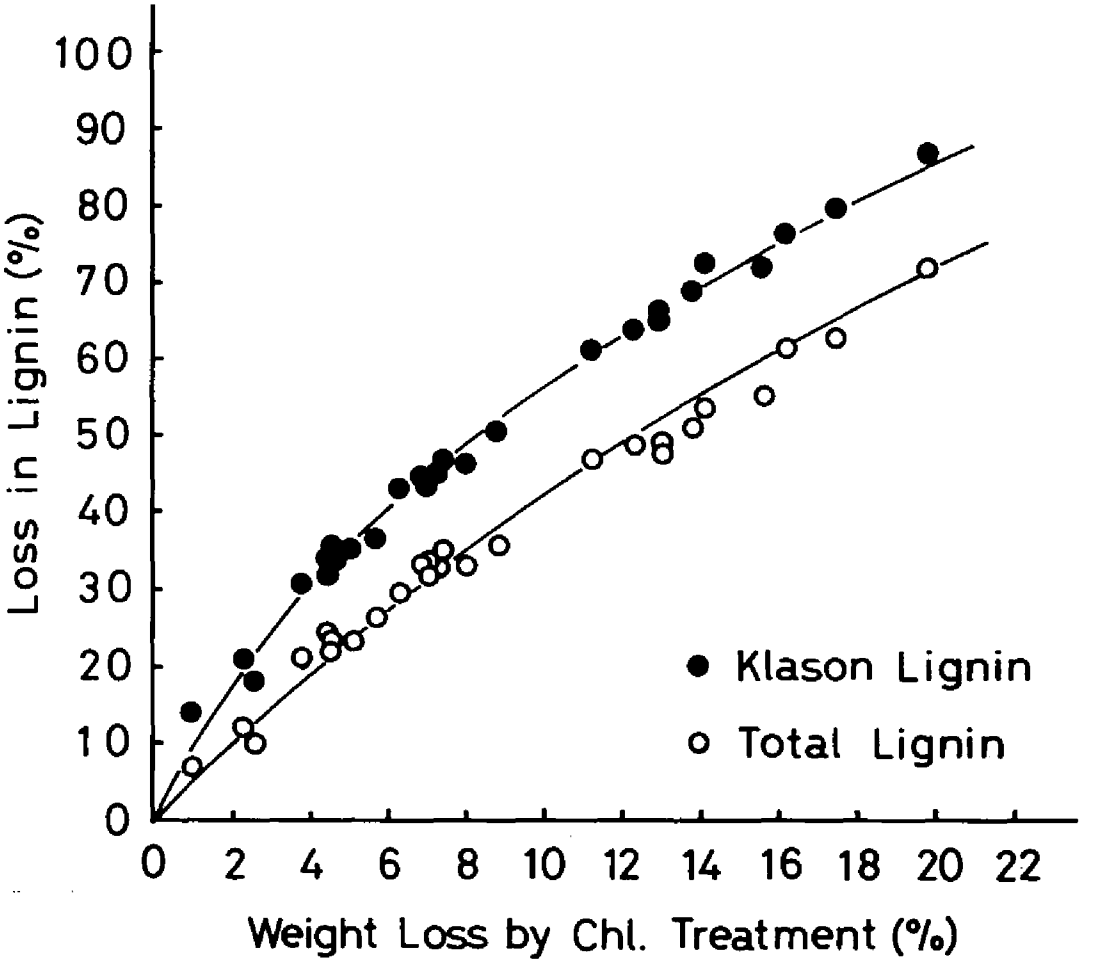



Fig. 46. Decrease of lignin in *Pinus densiflora* during chlorite treatment.

weight loss by chlorite treatment, which was equivalent to 20 % of delignification (Table 31) based on the assumption that effect of particle size is negligible in the chlorite treatment, and reached the maximum during 10 to 14 % of weight loss (43 to 55 % of delignification). However, in the case of *P. densiflora* attacked by *C. versicolor* (Fig. 50),

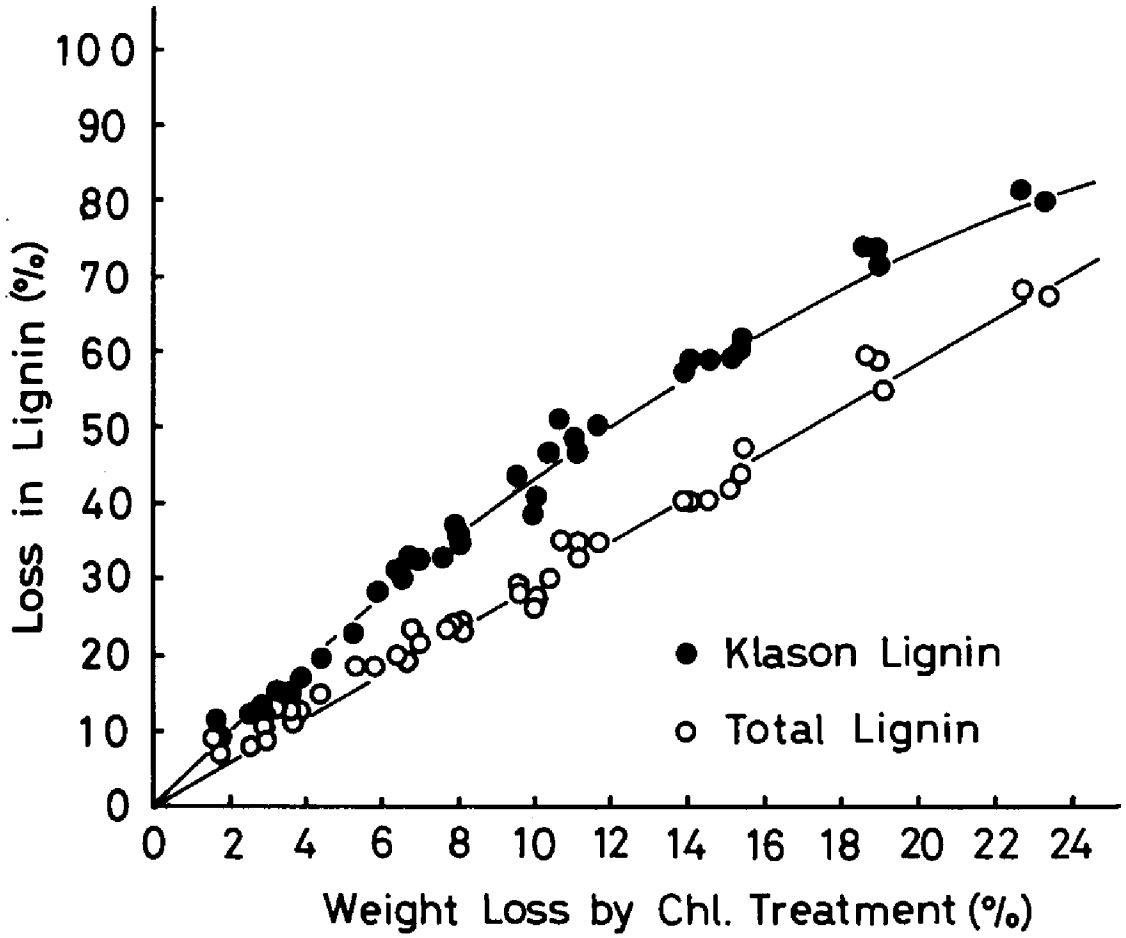



Fig. 47. Decrease of lignin in *Cryptomeria japonica* during chlorite treatment.

the curve was gentle but reached the maximum at greater stage of delignification. In the case of *C. japonica*, the acceleration of decay was nearly reverse for the two fungi. At the first 4 % of weight loss, equivalent to 12 % of delignification (Table 31), the acceleration of decay was about 30 % for *C. versicolor* but only about 10 % for *C. glo-*

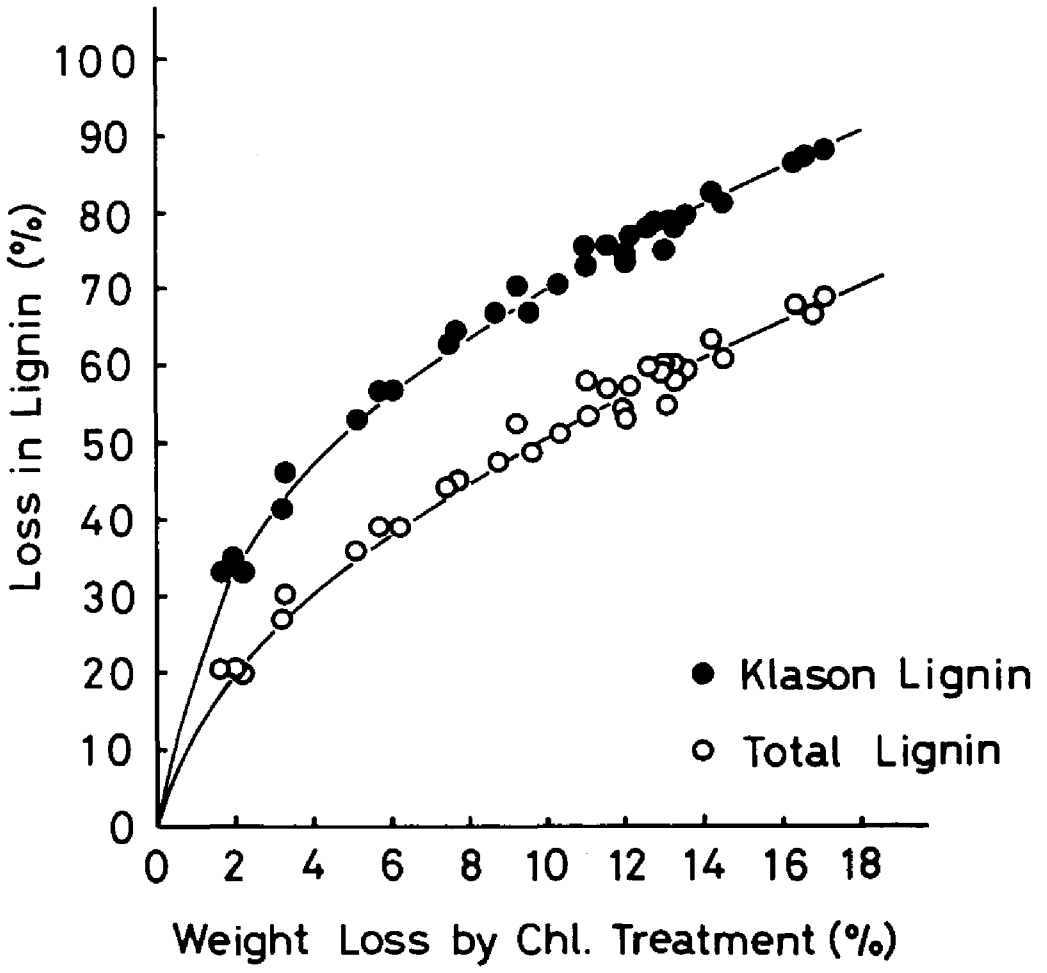



Fig. 48. Decrease of lignin in *Fagus crenata* during chlorite treatment.

*bosum*. However, the curve for *C. versicolor* reached the maximum level during the middle stage of delignification, whereas the curve for *C. globosum* still considerably steep at over 40 % of delignification.

In cases of the two softwoods exposed to *P. coccineus* (Fig. 51), the acceleration of decay was also found significantly at the first stage of delignification, although maximum levels in both woods for this

Table 31. The losses in total lignin content at different stages of chlorite treatment.

| Weight loss in wood (%)* | Loss in total lignin (%) | | | | | |
|---|---|---|---|---|---|---|
| | Smmothed | | | Estimated | | |
| | PD | CJ | FC | PD | CJ | FC |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 11 | 7 | 20 | 7 | 6 | 7 |
| 4 | 20 | 12 | 31 | 14 | 12 | 14 |
| 6 | 29 | 18 | 39 | 21 | 18 | 21 |
| 8 | 36 | 24 | 46 | 28 | 23 | 28 |
| 10 | 43 | 30 | 53 | 35 | 29 | 35 |
| 12 | 49 | 35 | 57 | 42 | 35 | 42 |
| 14 | 55 | 41 | 62 | 49 | 41 | 49 |
| 16 | 60 | 47 | 66 | 56 | 47 | 56 |
| 18 | 67 | 53 | 70 | 63 | 53 | 63 |
| 20 | 72 | 59 | — | 70 | 59 | 70 |
| 22 | — | 65 | — | 77 | 65 | 77 |

* $\frac{W_2 - W_3}{W_2} \times 100$ $W_2$ and $W_3$ are same as those in Table 30.

PD: *Pinus densiflora*, CJ: *Cryptomeria japonica*,
FC: *Fagus crenata*.

fungus were lower than those for *C. globosum* and *C. versicolor*.

In cases of both woods exposed to the two brown rot species, *S. lacrymans* (Fig. 52) and *C. puteana* (Fig. 53), the acceleration was undetected (*P. densiflora*) or only slight (*C. japonica*). The differences of weight losses between untreated and treated woods of *C. japonica* was much less for the two brown rot species.

In all cases of *F. crenata* (Figs. 49-53), the acceleration was

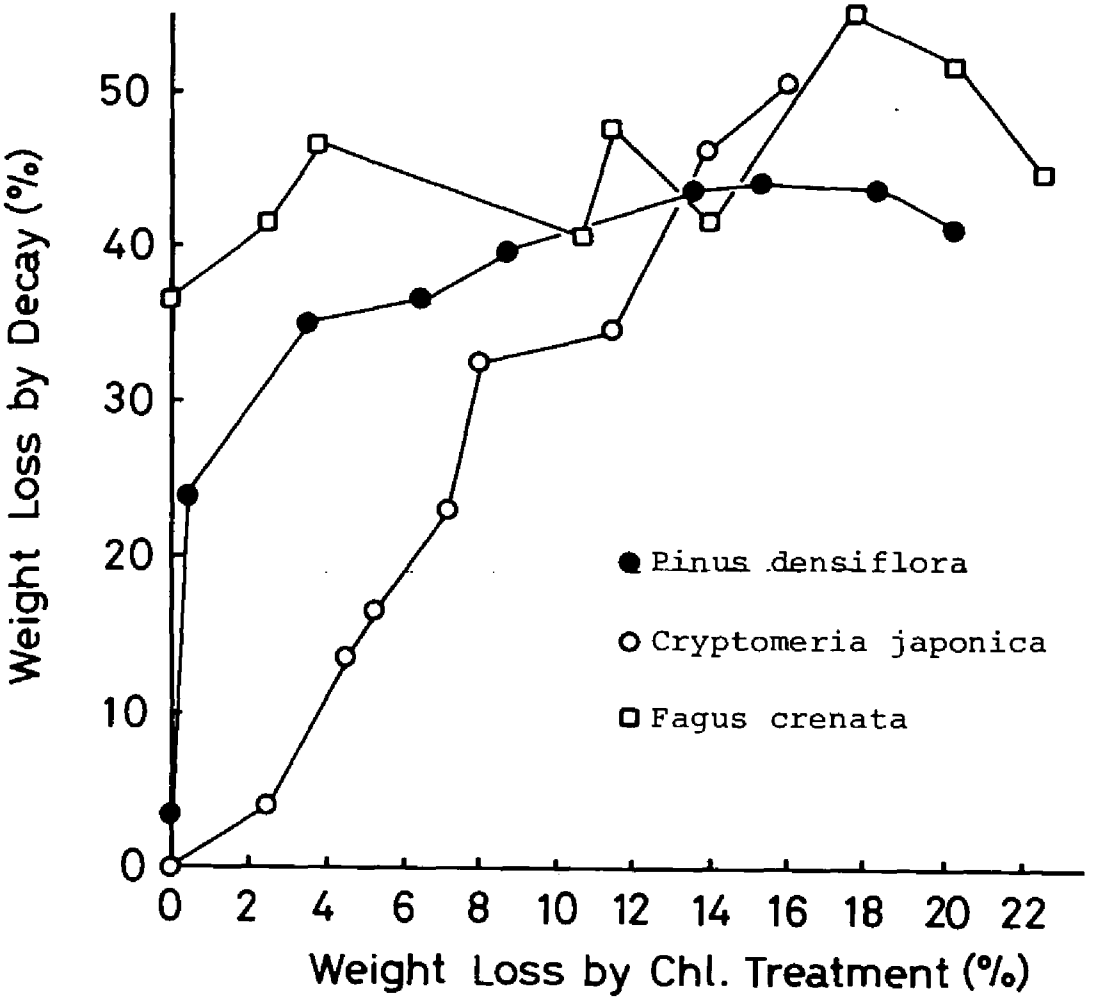



Fig. 49. Weight loss in wood exposed to *Chaetomium globosum* after different degrees of delignification.

significant at some stage of delignification. However, the extent of acceleration for this wood was generally smaller than that for the two softwoods. This suggests that the delignification treatment is less effective for the fungal attack of the originally susceptible wood such as *F. crenata*.

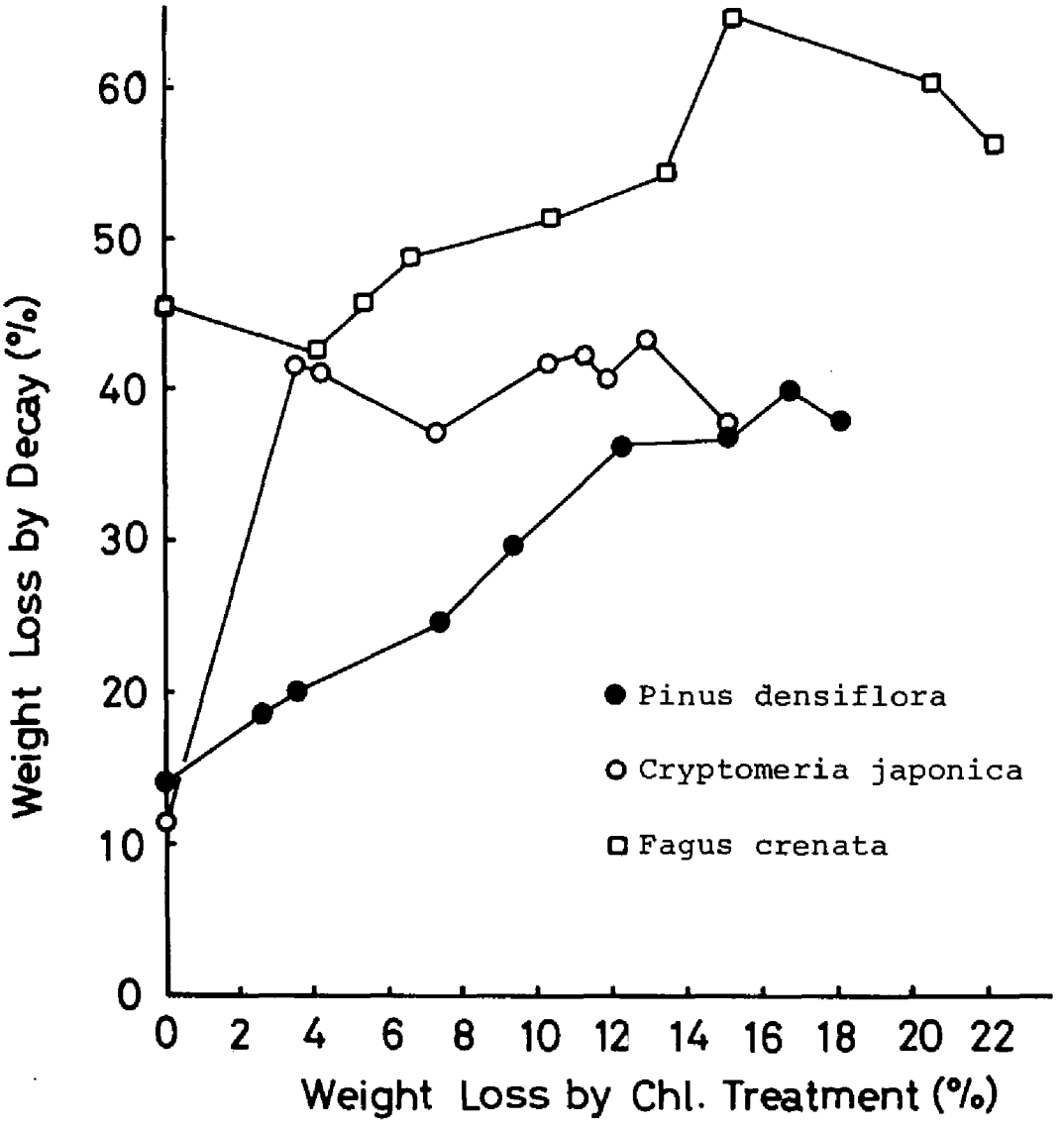



Fig. 50. Weight loss in wood exposed to *Coriolus versicolor* after different degrees of delignification.

Duncan[37] found that leaching of redwood (*Sequoia sempervirens*) with sodium hypochlorite solution (containing 2 ppm of available chlo-

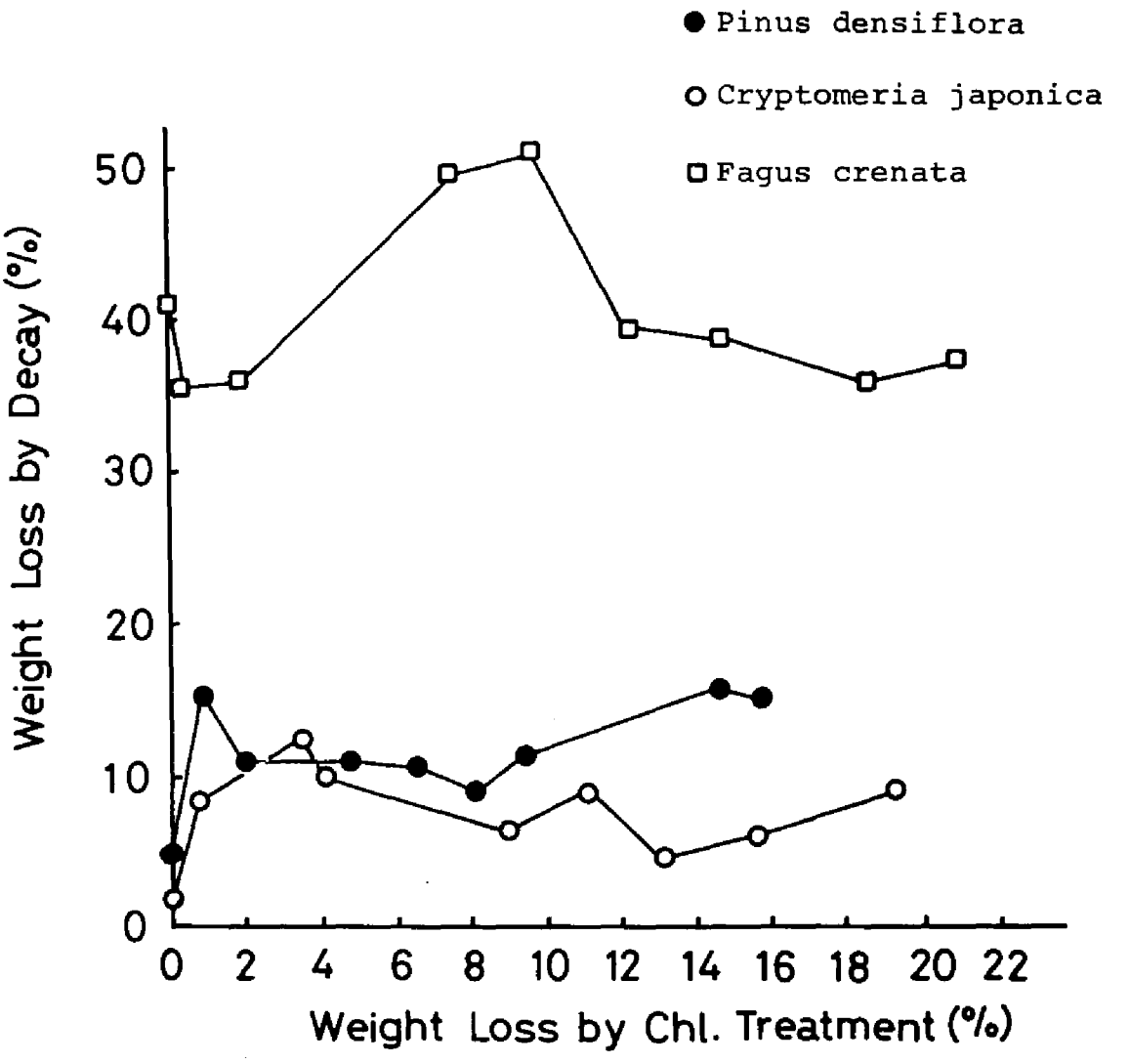



Fig. 51. Weight loss in wood exposed to *Pycnoporus coccineus* after different degrees of delignification.

rine) facilitated attack by many isolates of soft rot fungi including *Chaetomium funicolum* and by a white rot fungus, *Poria nigrescens*, but

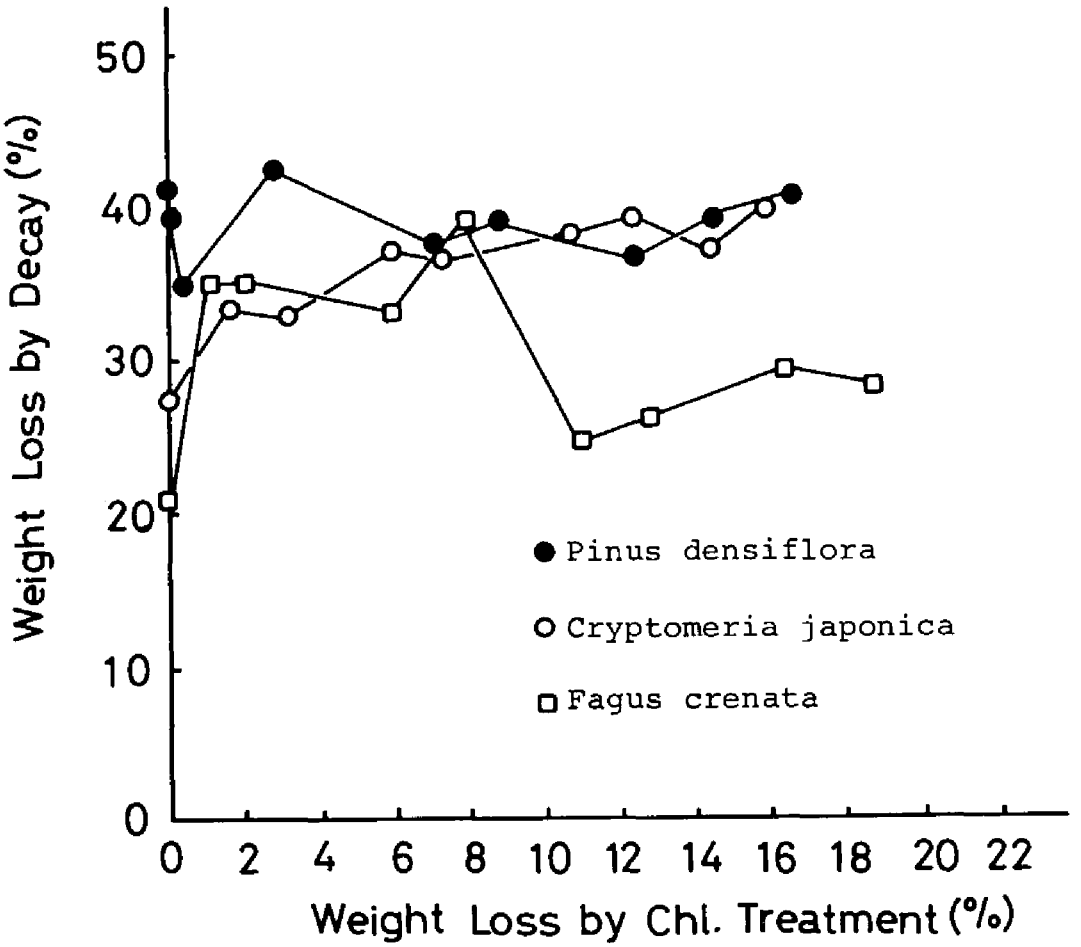



Fig. 52. Weight loss in wood exposed to *Serpula lacrymans* after different degrees of delignification.

it had no apparent effect on the wood-attacking ability of a brown rot fungus, *Poria oleraceae*. Bailey et al.[8] reported that partial delignification of softwoods (*Picea abies* and *Pinus sylvestris*) with acidified sodium chlorite accelerated attack by soft rot fungi, *C. globosum*, *Ceratocystis* sp. and *Paecilomyces* sp., but it was not effective for

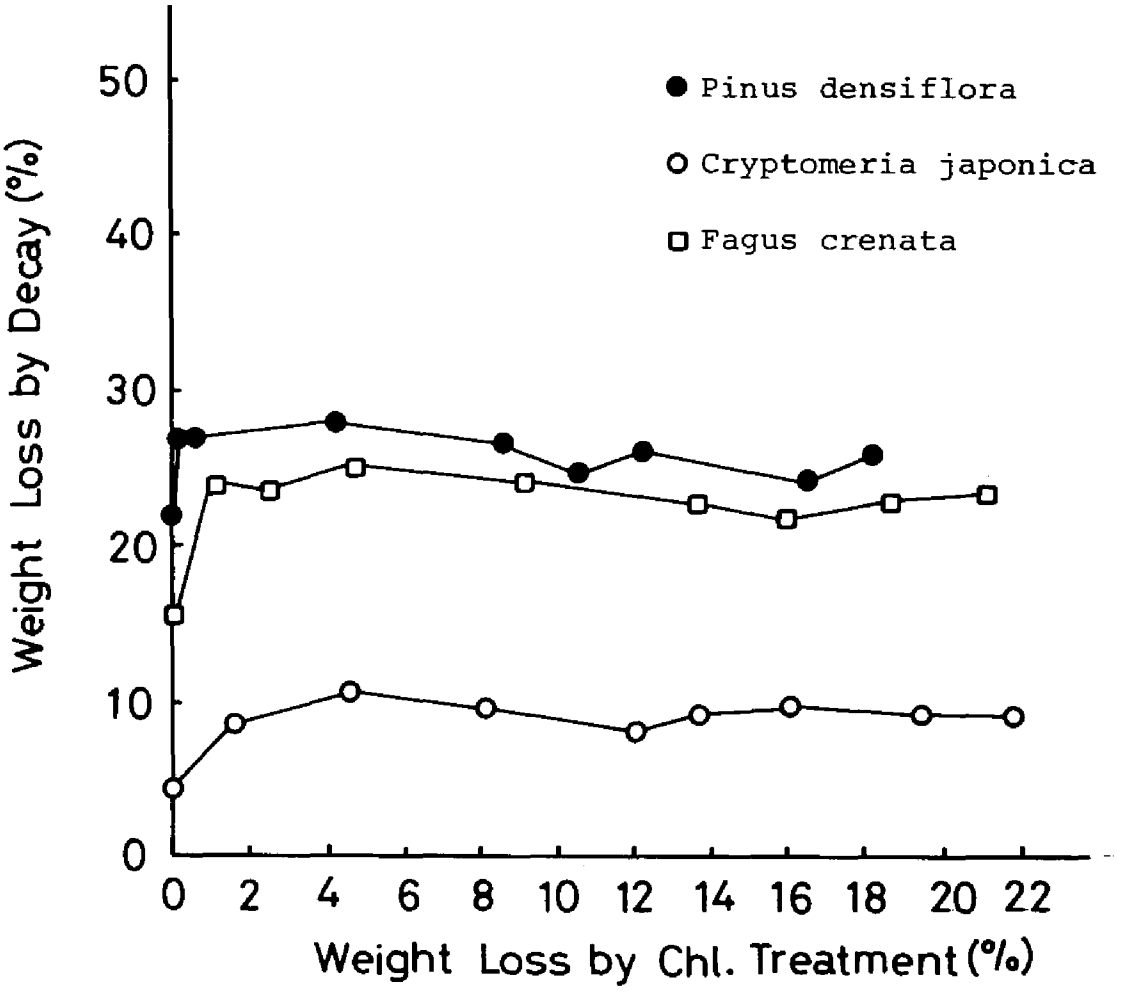



Fig. 53. Weight loss in wood exposed to *Coniophora puteana* after different degrees of delignification.

attack by brown rot fungi, *Coniophora cerebella*, *Poria vaporaria* and *Polyporus schweinizii*. Nouvertné[98] obtained similar results on the extent of degradation of delignified softwoods by three species of soft rot fungi. In their investigations, loss of lignin by the delignification treatment was not determined experimentally and only estimated

from the original lignin content found in a literature. Furthermore, the observations were done only at one or few stages of delignification. In this experiment, effect of the chlorite delignification was examined at various stages of delignification. Similar results were obtained here again and then it can be concluded that the delignification treatment of softwoods is effective for acceleration of wood-attacking capacity of soft rot- and white rot fungi but not for that of brown rot fungi.

Although white rot fungi are regarded as lignin-degrading fungi, they have a preference for hardwoods containing smaller amount of lignin than softwoods. It can be assumed that white rot fungi have two types of enzyme system. The first is involved in breakdown of polysaccharides, and the second is in the breakdown of lignin. Brown rot- and soft rot fungi have certainly the first system only, or have the first and the incomplete system of the second. White rot fungi are regarded as an advanced group, evolved out of a primitive group which lacks extracellular oxidase and prefers softwoods to hardwoods[97]. If the second system which is associated with lignin degradation is developed gradually in the course of evolution, this system may be still minor for the survival of white rot fungi in the natural habitat compared with the first system. Fukuda and Haraguchi[48] and Kirk et al.[72] presented that the lignin-degradaing ability of white rot fungi is an effective means to gain access to the cellulose in lignified cell wall but does not serve effectively to metabolize lignin itself as energy- and nutrient sources. According to this theory, removal of lignin from cell wall may facilitate the action of cellulolytic enzyme system in the attack of softwoods

by white rot fungi. Such an accelerative effect was found here for the two softwoods exposed to the white rot fungi used.

Brown rot fungi have a limited ability to degrade lignin, although they are associated most frequently with the decay of softwoods containing higher amount of lignin than hardwoods. However, delignification treatment of softwoods had no effect on the wood-attacking ability of the brown rot fungi used. Koenigs[76] found that low concentrations of $H_2O_2$ and $Fe^{++}$ caused rapid weight loss of sweetgum (*Liquidamber styraciflua*) and loblolly pine (*Pinus taeda*) and that the degree of polymerization of cellulose in treated woods decreased rapidly at low weight loss and then diminished gradually. This effect was similar to that in wood created by brown rot fungi[33]. Out of this fact and the evidence with production of $H_2O_2$ from native substrates in wood by brown rot fungi[77], he proposed that these fungi might attack cellulose and partly decay wood via an $H_2O_2$-$Fe^{++}$ system. This suggests that brown rot is differentiated from white rot and soft rot by the rather oxidative system for the decomposition of cellulose in wood.

Acceleration of attacking capacity on softwoods by delignification was evidenced in both soft rot- and white rot fungi, but acceleration pattern varied apparently with wood and fungal species. It can be considered, therefore, that in the rapid and shorter acceleration (*P. densiflora* vs. *C. globosum* and *C. japonica* vs. *C. versicolor*) a modification of lignin may play an important role, and that a removal of lignin may act as a main agent in the slow and longer acceleration (*P. densiflora* vs. *C. versicolor* and *C. japonica* vs. *C. globosum*). With respect to

this consideration, it will be necessary to deal with the analyses of substances remaining in the wood after the chlorite treatment and exposure to fungal attack.

## 4-3 Summary

Effects of some biological- and chemical treatments of softwoods (*Pinus densiflora* and *Cryptomeria japonica*) on the wood-decaying capacity of *Chaetomium globosum* were investigated. For comparison, data are included on a hardwood, *Fagus crenata*, and on white rot- and brown rot fungi. Pre-exposure of woods to 9 Basidiomycotina and 1 Deuteromycotina did not facilitate the attack by *C. globosum*. Lower attacking capacity of this fungus on softwoods was not accelerated by treatments with both mild alkali and acid. However, treatment of softwoods with acidified sodium chlorite was very effective for acceleration of attacking capacity of *C. globosum* and white rot fungus, *Coriolus versicolor*, but not for that of brown rot fungi, *Coniophora puteana* and *Serpula lacrymans*. Acceleration of decay was found in all cases of *F. crenata*, but the extent was generally smaller than that for the two softwoods. Acceleration pattern found in softwoods varied with wood and fungal species. It is proposed that in the rapid and shorter acceleration (*P. densiflora* vs. *C. globosum* and *C. japonica* vs. *C. versicolor*) a modification of lignin may play an important role, and that a removal of lignin may act as a main agent in the slow and longer acceleration (*P. densiflora* vs. *C. versicolor* and *C. japonica* vs. *C. globosum*).

# CHAPTER 5 SIGNIFICANCE OF LIGNIN IN DECAY RESISTANCE OF WOOD AGAINST CHAETOMIUM GLOBOSUM

## 5-1 Removal of lignin from non- and partially delignified woods by *C. globosum*[123]

As has been mentioned in Chapter 4, attacking capacity of soft rot- and white rot fungi on softwoods was greatly accelerated by the treatment of partial delignification, whereas that of brown rot fungi was not by such a treatment. Lignin-degrading ability of white rot fungi is widely known, and is recently regarded as an effective means to gain access to the cellulose in lignified cell wall by some investigators[48,72]. Soft rot fungi are similar to brown rot fungi on their lower ability to degrade lignin, but they do not apparently have a "pre-cellulolytic system"[8], as brown rot fungi do. The system has been evidenced partly by the production of $H_2O_2$[76,77] but has not been fully characterized.

In this chapter, the pattern of lignin removal from partially delignified woods by a soft rot fungus, *Chaetomium globosum* Kunze (IAM 8059), and a white rot fungus, *Coriolus versicolor* Quél. (FES 1030), has been studied on two softwoods, *Pinus densiflora* Sieb. et Zucc. and *Cryptomeria japonica* D. Don, and one hardwood, *Fagus crenata* Blume, with reference to the different acceleration pattern of wood decay described in Chapter 4. On the basis of the results obtained, significance of lignin in different decay resistance of wood against *C. globosum* has been discussed, comparing with that against *C. versicolor*.

## Materials and Methods

### *Preparation of samples*

Wood blocks, subjected to the chlorite treatment and fungal attack (see Chapter 4), were rated into six to ten groups according to weight losses caused by chlorite treatment before decay. Weights of respective groups after each of three treatments (extraction with ethanol-benzene, delignification and exposure to fungal attack) were calculated from the weight of each block determined after each treatment. Each group was separately ground to pass a 40 mesh sieve and throughly air dried.

### *Lignin determination*

The Klason lignin content was determined by the JIS P 8008-1961. The acid-soluble lignin content was determined on the hydrolysate from the Klason lignin by the same procedures described in Chapter 4. The total lignin content was calculated as insoluble Klason lignin plus the acid-soluble lignin estimated spectrophotometrically.

## Results and Discussion

Table 32 shows the loss of weight by chlorite treatment and fungal attack of each group for lignin analysis. As described in Chapter 4, rapid and shorter acceleration of fungal attack characterizes the cases of *P. densiflora* attacked by *C. globosum* and *C. japonica* by *C. versicolor*. Slow and longer acceleration characterizes *P. densiflora* attacked by *C. versicolor* and *C. japonica* by *C. globosum*. However, in the case of *F. crenata*, acceleration was not greater than in the two softwoods, but was rather slow and longer with respect to these fungi.

Table 32. The loss of weight in the samples for lignin analysis caused by chlorite treatment and exposure to fungal attack.

| Wood / Fungus | *Pinus densiflora* | | *Cryptomeria japonica* | | *Fagus crenata* | |
|---|---|---|---|---|---|---|
| | Weight loss by chlorite treatment(%) | Weight loss by decay(%) | Weight loss by chlorite treatment(%) | Weight loss by decay(%) | Weight loss by chlorite treatment(%) | Weight loss by decay(%) |
| *Chaetomium globosum* | 0* | 3.50 | 0* | 0.00 | 0* | 36.50 |
| | 0.46 | 24.11 | 2.53 | 3.83 | 2.52 | 40.45 |
| | 3.56 | 34.74 | 4.50 | 13.67 | 3.75 | 46.70 |
| | 6.48 | 36.35 | 5.28 | 16.31 | 10.64 | 40.62 |
| | 8.70 | 39.24 | 7.21 | 22.93 | 14.02 | 41.44 |
| | 13.61 | 43.66 | 7.96 | 32.21 | 17.81 | 54.81 |
| | 15.41 | 43.78 | 11.37 | 34.35 | | |
| | 18.37 | 43.64 | 13.88 | 45.91 | | |
| | 20.29 | 40.89 | 16.05 | 50.53 | | |
| *Coriolus versicolor* | 0* | 14.00 | 0* | 11.50 | 0* | 45.50 |
| | 2.59 | 18.61 | 3.48 | 41.67 | 4.17 | 42.70 |
| | 3.56 | 19.91 | 4.21 | 41.32 | 5.40 | 45.78 |
| | 7.58 | 24.68 | 7.35 | 37.20 | 6.56 | 48.71 |
| | 8.49 | 28.82 | 10.26 | 41.23 | 10.77 | 56.75 |
| | 9.45 | 29.65 | 10.79 | 39.67 | 13.48 | 52.58 |
| | 12.35 | 40.91 | 11.31 | 42.17 | 15.26 | 63.97 |
| | 15.09 | 36.39 | 11.85 | 40.37 | | |
| | 17.00 | 39.35 | 12.92 | 42.92 | | |
| | 18.06 | 32.28 | 15.06 | 37.45 | | |

All values are expressed on the basis of the weight of extractive-free wood before chlorite treatment. 0*: Extracted with ethanol-benzene, not treated with sodium chlorite and acetic acid.

Table 33. Klason and acid-soluble lignin contents of sound woods*.

| Wood | Lignin content (%) | | |
|---|---|---|---|
| | Klason | Acid-soluble | Total |
| *Pinus densiflora* | 28.33 | 0.26 | 28.59 |
| *Cryptomeria japonica* | 33.67 | 0.37 | 34.04 |
| *Fagus crenata* | 26.47 | 2.14 | 28.61 |

* An average of the values from three separate Klason hydrolyses of 0.3 mm thick wood shavings.

Figs. 54-56 show the results with the loss of lignin from each group of the three wood species subjected to chlorite treatment and fungal attack. Each point is an average of the data from two separate experiments. Percent of lignin loss is expressed on the basis of the original amount of lignin in sound wood shown in Table 33. Dotted line on each figure represents the pattern of lignin removal from 0.3 mm thick shavings of each wood species during chlorite treatment (see Figs. 46-48). By comparing the pattern of lignin removal during chlorite treatment for different forms of the wood sample of *Picea mariana*, Ahlgren and Goring[2] concluded that the effect of particle size was negligible in the entire range studied (0.04 to 2.0 mm thick in the longitudinal direction). On the assumption that size effect is similarly negligible or very weak between 0.3 mm thick shavings and 5.0 mm blocks used in the present experiment, differences between solid and dotted lines at a certain point on the ordinate shows a rough estimate of the lignin loss caused by fungal attack.

Figs. 57-59 show the ratio of lignin loss to weight loss in each

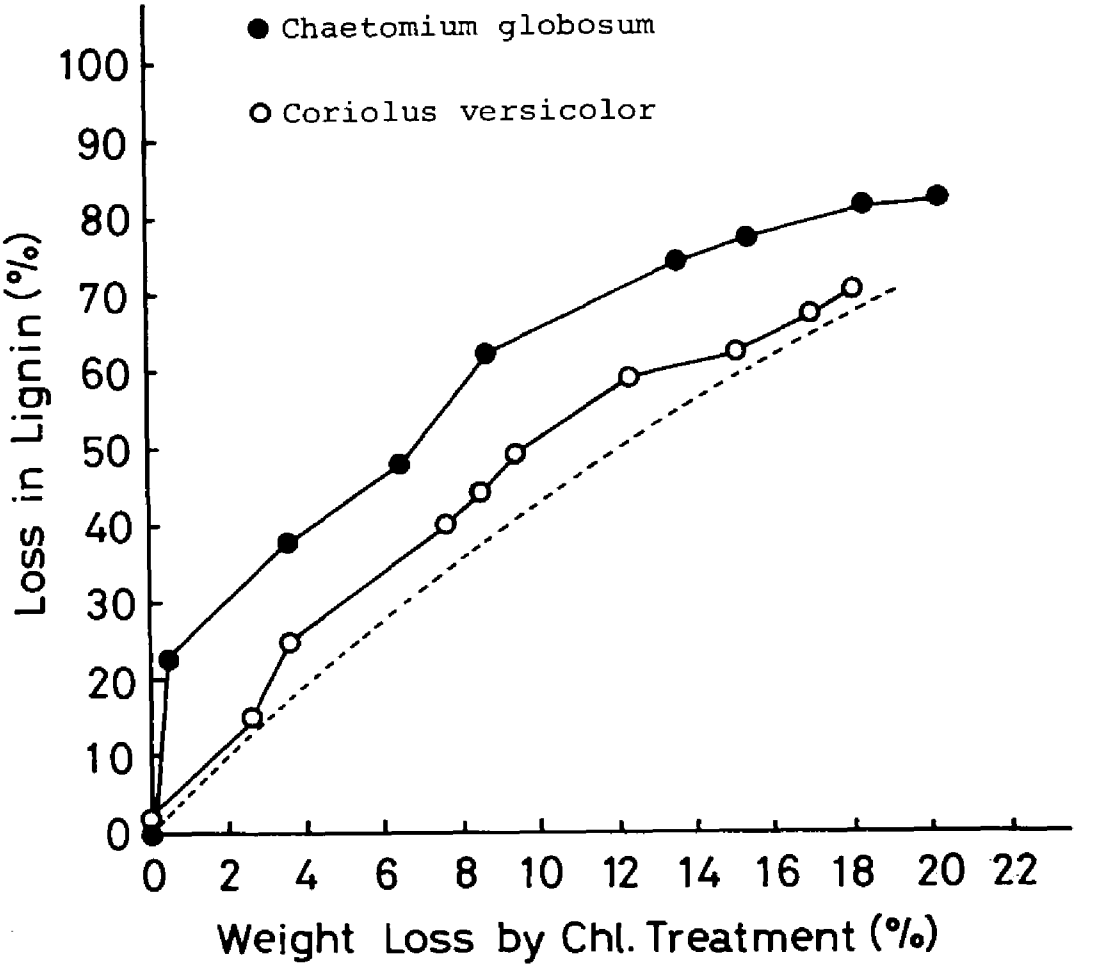



Fig. 54. Decrease of lignin in *Pinus densiflora* during chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of lignin removal from wood shavings of *P. densiflora* during chlorite treatment.

group of the three wood species exposed to fungal attack after chlorite treatment. The ratio was calculated by dividing difference between solid and dotted lines in Figs. 54-56 by corresponding percent of weight loss in Table 32. The ratio reaches 1.0 when the lignin and non-lignin compo-

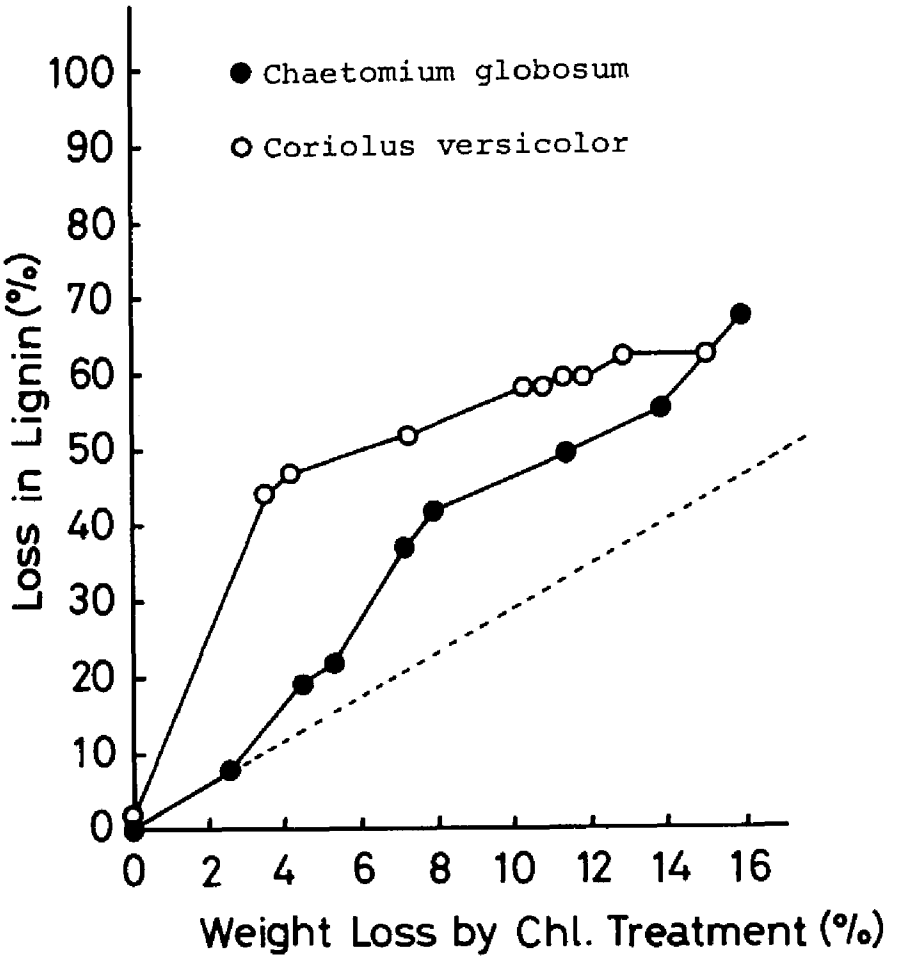



Fig. 55. Decrease of lignin in *Cryptomeria japonica* during chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of lignin removal from wood shavings of *C. japonica* during chlorite treatment.

nents (carbohydrates) are removed at the same relative rates from wood by fungus.

Figs. 60-62 show the data on the ratio of acid-soluble lignin to total lignin in each group of the three wood species. The ratio was

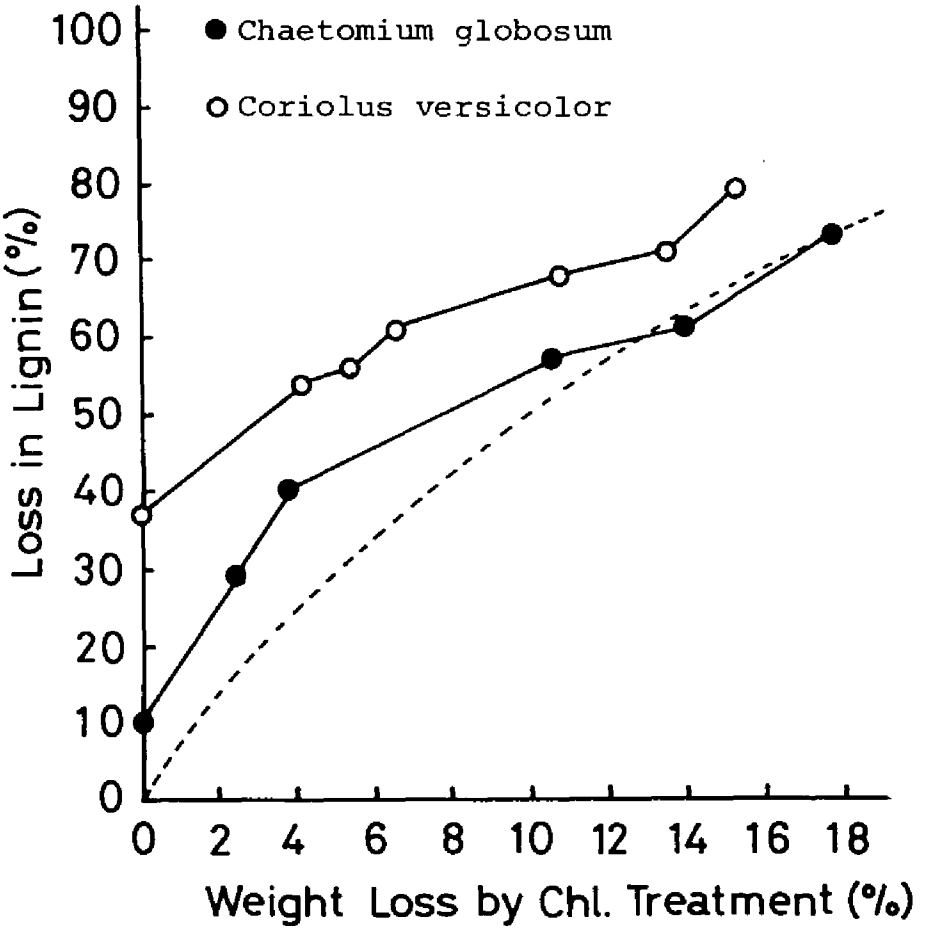



Fig. 56. Decrease of lignin in *Fagus crenata* during chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of lignin removal from wood shavings of *F. crenata* during chlorite treatment.

calculated by dividing percent of acid-soluble lignin by percent of total lignin. Percent of the two kinds of lignin was based on the weight of wood after decay. Dotted line represents the same ratio in wood shavings of each species after chlorite treatment.

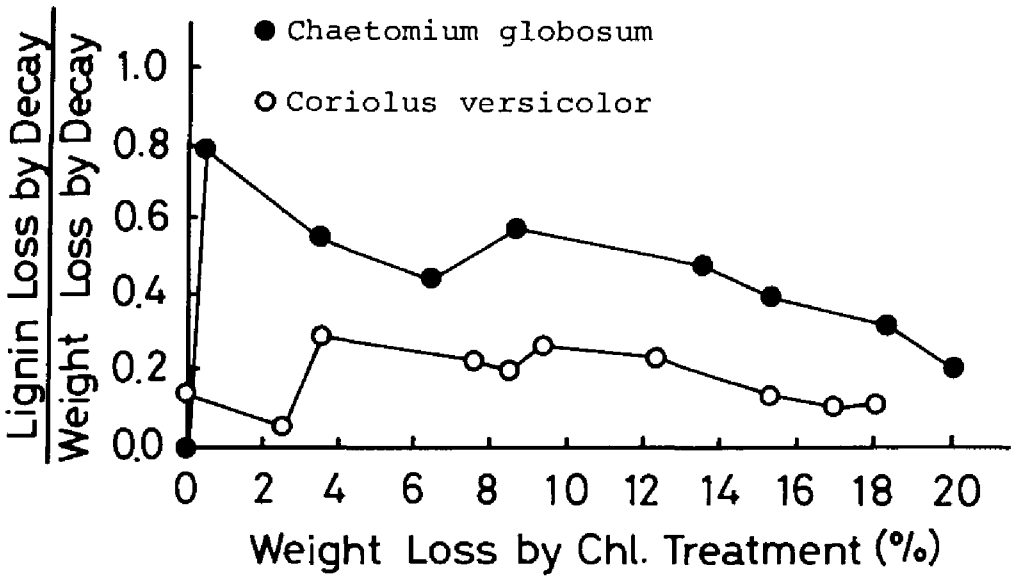



Fig. 57. The ratio of lignin loss to weight loss caused by fungal attack on chlorite treated wood of *Pinus densiflora*.

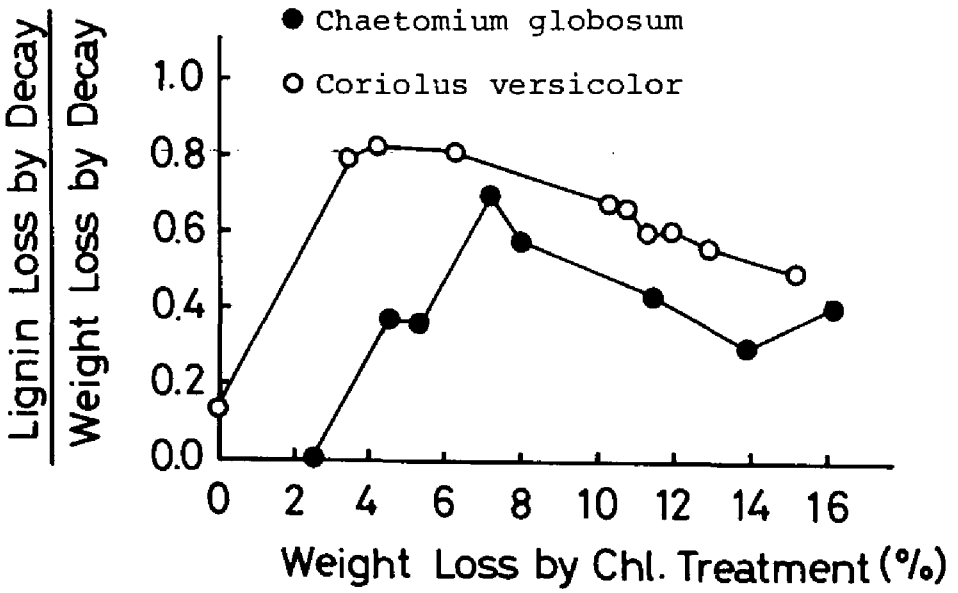



Fig. 58. The ratio of lignin loss to weight loss caused by fungal attack on chlorite treated wood of *Cryptomeria japonica*.

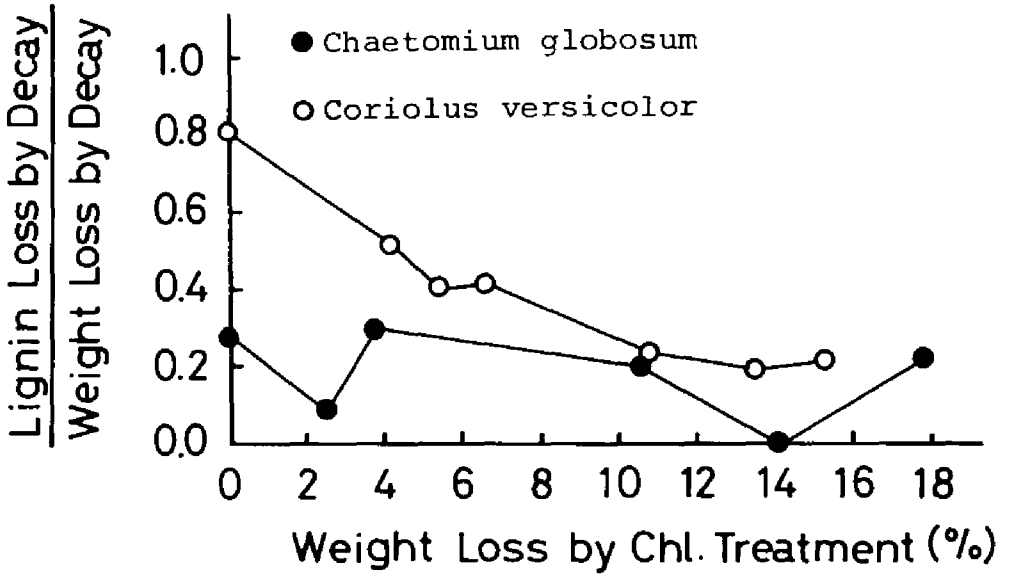



Fig. 59. The ratio of lignin loss to weight loss caused by fungal attack on chlorite treated wood of *Fagus crenata*.

A considerable amount of lignin was removed from *P. densiflora* by *C. globosum* during the first 0.46 % of weight loss by chlorite treatment (Fig. 54) and the ratio of lignin loss to weight loss was highest at this stage (Fig. 57). Removal of lignin from *P. densiflora* by *C. versicolor* was slower than by *C. globosum* throughout all the stages. That partly reflected the lower acceleration of wood decay by the former. However, the ratio of lignin loss to weight loss for *C. versicolor* was also smaller than for *C. globosum* at every stage of delignification with an exception at 0 % of weight loss (non-chlorite treatment). On the other hand, the ratio of acid-soluble lignin to total lignin for *C. versicolor* was always larger than for *C. globosum* (Fig. 60), which suggests that solubilized lignin derived from insoluble Klason lignin was con-

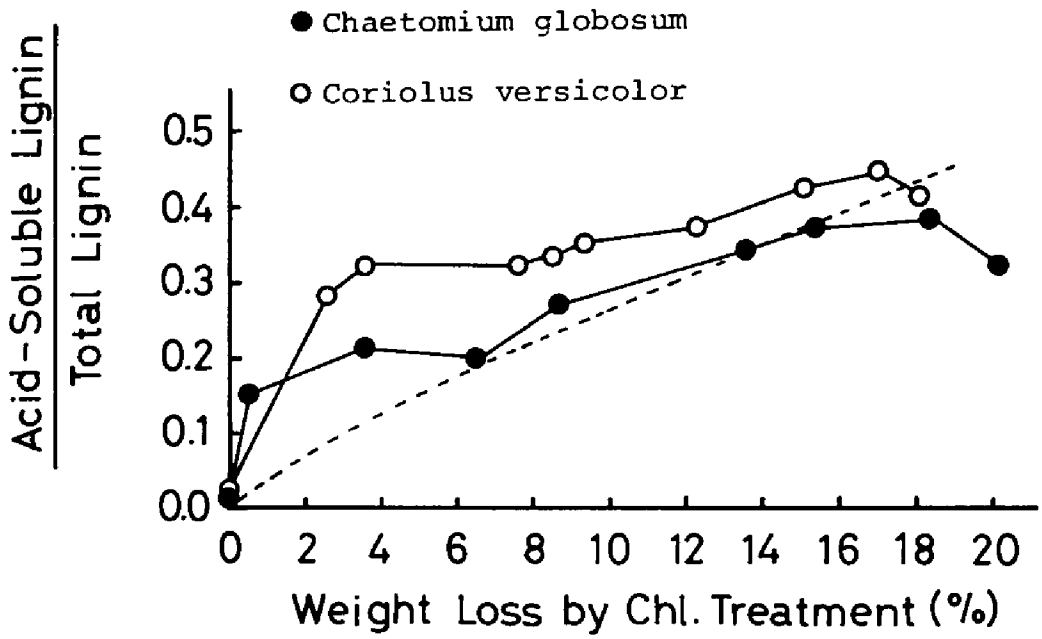



Fig. 60. The ratio of acid-soluble lignin to total lignin in *Pinus densiflora* after chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of accumlation of acid-soluble lignin in wood shavings of *P. densiflora* during chlorite treatment.

centrated because of the lesser action of *C. versicolor* on this substance.

In the case of *C. japonica*, the pattern of lignin removal was nearly reverse for the two fungi. During the first 3.48 % of weight loss by delignification, large amount of lignin was removed by *C. versicolor* (Fig. 55). The slower rate of lignin removal by *C. globosum* was coincident with the slower acceleration of wood decay by the fungus. The ratio of lignin loss to weight loss for *C. versicolor* reached the maxi-

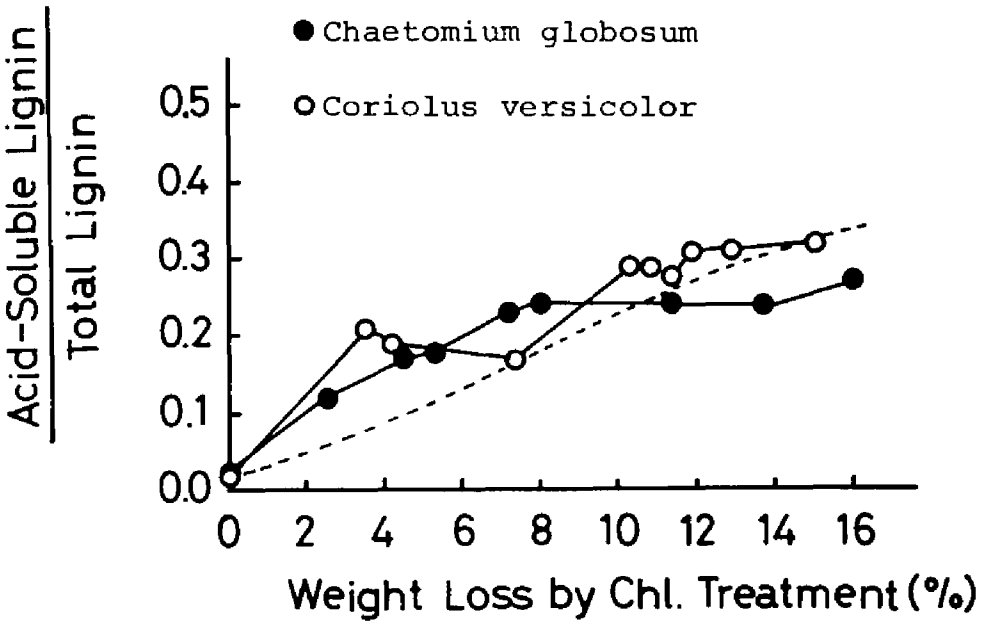



Fig. 61. The ratio of acid-soluble lignin to total lignin in *Cryptomeria japonica* after chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of accumlation of acid-soluble lignin in wood shavings of *C. japonica* during chlorite treatment.

mum level at about 4 % of weight loss by delignification (Fig. 58). The ratio for *C. globosum* reached maximum level at about 7 % of weight loss, and was always smaller than for *C. versicolor*. The ratio of acid-soluble lignin for *C. globosum* was smaller than that for *C. versicolor* in a range of over 10 % of weight loss, and that for non-decayed wood shavings at greater extent of weight loss (Figs. 60 and 61). This suggests that the acid-soluble lignin was rapidly depleted by *C. globosum* at these stages.

Although a considerable amount of substances was removed from non-

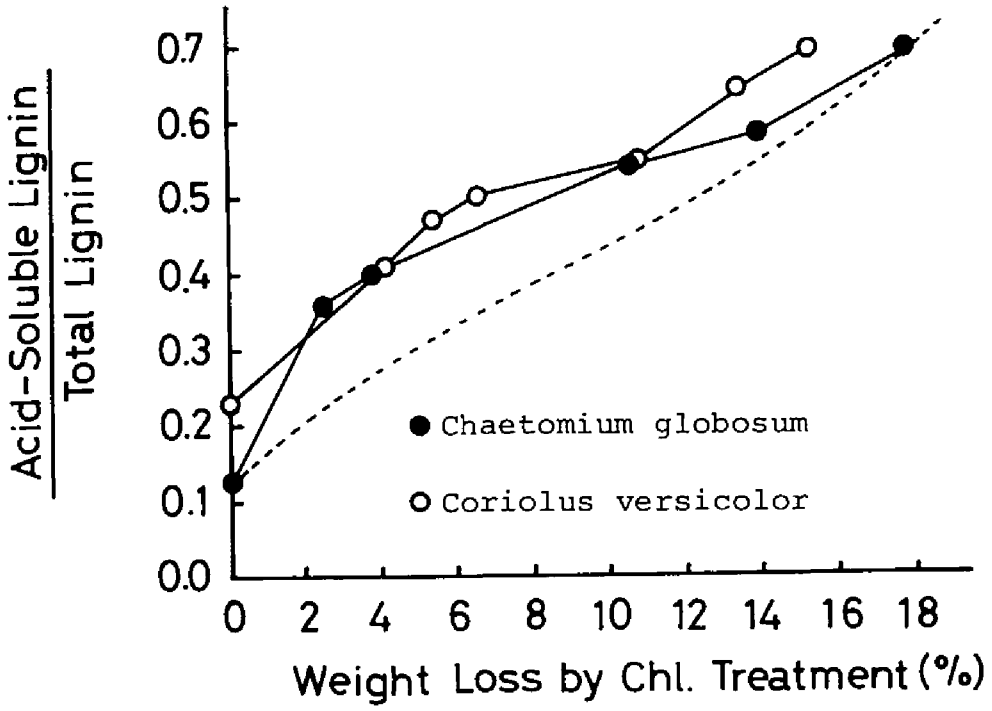



Fig. 62. The ratio of acid-soluble lignin to total lignin in *Fagus crenata* after chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of accumlation of acid-soluble lignin in wood shavings of *F. crenata* during chlorite treatment.

delignified softwoods by *C. versicolor* (Table 32), no removal of lignin was detected (Figs. 54 and 55). As shown in Figs. 60 and 61, the ratio of acid-soluble lignin for *C. versicolor* was nearly equal to the sound wood. These results assume that the lignin remaining in non-delignified softwoods is mostly unaltered. This suggestion extends to the cases of the two non-delignified softwoods exposed to *C. globosum*. It is well known that white rot fungi metabolize all the major constituents of lignified cell walls - the cellulose, hemicelluloses and lignin. How-

ever, various species of white rot fungi differ in relative rates at which they remove the major componets[70]. Sometimes a distinction is made between white rot fungi and simultaneous rot fungi. The former decompose wood successively, beginning with lignin and hemicelluloses and deteriorating the cellulose only at a later stage, while the latter decompose all substances of the lignified cell wall simultaneously[61]. Differences between the two types have been found by fluorescense microscopy[5] and chemical investigations of wall degradation[53,86,92,133]. However, it seems that these differences occasionally fluctuate with wood species[5] and that these types are not phylogenetically fixed characters. Seifert[109], in the analysis of chemical changes during decay processes, considered that white rot and simultaneous rot are synonymous. A white rot fungus, *C. versicolor*, used in this investigation, is often recognized as an example of a group which removes the three major components approximately simultaneously[33,65]. As shown in Table 32, Fig. 56 and Fig. 59, loss of lignin and the ratio of lignin loss to weight loss in non-delignified wood of *F. crenata* attacked by *C. versicolor* apparently demonstrate that wood constituents are removed at approximately the same relative rates. It seems therefore that such a simultaneous removal of the major constituents occurs only in hardwoods but a preferential degradation of non-lignin components sometimes occurs in non-lignified or original softwoods.

The slower rate of lignin removal and the lower ratio of lignin loss in non-delignified wood of *F. crenata* attacked by *C. globosum* (Figs. 56 and 59) agreed with the results obtained by Savory and Pinion[105]

and Levi and Preston[81]. Soft rot fungi do not attack softwoods so rapidly or extensively as they do hardwoods. A large number of white rot fungi, including *C. versicolor* and *Pycnoporus coccineus*, prefer hardwoods to softwoods. Hence, it is possible to assume that the lignin in softwoods is more or less hindrance in both types of wood-decaying fungi. Of the two types of acceleration pattern of decay observed in the two softwoods, the rapid and shorter acceleration was accompanied with the rapid rate of lignin removal and the higher ratio of lignin loss to weight loss during first stage of delignification. In such a case, hindrance by lignin may be rather qualitative than quantitative, so that a certain modification of lignin which was caused by the chlorite treatment for a short time seems to act as a trigger for succeeding degradation of the modified lignin. On the other hand, the slow and longer acceleration of decay was accompanied with slow or poor overall removal of lignin. In the case of *P. densiflora* attacked by *C. versicolor*, the lignin was removed at apparently slower rate than non-lignin components, and removal or modification of lignin acts largely for facilitating to gain access to the carbohydrates. In such a case, hindrance by lignin may act rather quantitatively. In the case of *C. japonica* attacked by *C. globosum*, however, hindrance by lignin may be rather qualitative at least during the first 7 % of weight loss by delignification, since the ratio of lignin loss to weight loss increased in proportion to the extent of delignification and reached the maximum level at about 7 % of weight loss. This suggests that the lignin is a greater qualitative hundrance in *C. globosum* for *C. japonica* than for *P. densi-*

*flora*.

Although *F. crenata* is highly susceptible to both fungi, the rate of lignin removal and the ratio of lignin loss to weight loss were always slower and smaller in *C. globosum* than in *C. versicolor*. If it is assumed that the lignin is also hindrance in both fungi even in this easily attackable hardwood, removal of lignin by chlorite treatment may help both fungi for reducing the hindrance. This hindrance-reducing system may be less operative as the amount of lignin decreases. This was confirmed in *C. versicolor* by the constant decrease in the ratio of lignin loss to weight loss but not in *C. globosum* (Fig. 59). In *F. crenata* attacked by *C. globosum*, the poor removal of lignin and the lower ratio of lignin loss to weight loss were demonstrated throughout the stages. From the results, it can be considered that the lignin is not hindrance in *C. globosum* in *F. crenata* and removal of lignin by chlorite treatment is less helpful for attack on the wood by the fungus.

In the investigations on the chemical changes of wood caused by wood-decaying fungi, lignin is analysed mostly by the sulfuric acid method and determined as insoluble Klason lignin. Acid-soluble lignin is scaecely determined and mostly included among other materials than major wood components. Eslyn et al.[42] demonstrated that the other materials considerably increased in proportion to the increase of weight loss. The amount of insoluble lignin is small in original sound woods (Table 33) but becomes increasingly large within a limited range of delignification and fungal attack. Soluble lignin is mostly regarded as the degraded or modified lignin caused by the chlorite treatment and

fungal attack. Therefore, the estimation should be made on determination of total lignin (insoluble Klason lignin plus the ultraviolet-estimated acid-soluble lignin) rather than that of insoluble Klason lignin only to obtain the preciser amount of lignin remaining in decayed wood, although the estimation by ultraviolet absorption can not be fully accurate.

The greater resistance of softwoods to soft rot attack has been attributed to a physical blocking of the enzymes by the higher lignin content[103]. When the extent of acceleration of decay by *C. globosum* for 44 softwood species subjected to a six-hour delignification was compared, it was noticeably proportional to the extent of delignification (see Figs. 41 and 42 in Chapter 4). This seems to support the proposal of physical blocking action by lignin. However, the acceleration was often detected at very early stage of delignification. As has been mentioned, the pattern of acceleration varied with wood and fungal species. From these and the results with the pattern of lignin removal by soft rot- and white rot fungi, it can be concluded that the lignin in softwoods is more or less hindrance in both types of wood rot fungi. With respect to a soft rot fungus, *C. globosum*, hindrance by lignin may be rather qualitative than quantitative, because of the rapid increase of the ratio of lignin loss to weight loss in both softwoods. For a white rot fungus, *C. versicolor*, however, hindrance by lignin may act qualitatively on *C. japonica* but quantitatively on *P. densiflora*. Quantitative hindrance is considered simply in terms of a physical blocking of the cellulolytic enzymes by lignin. Qualitative hindrance seems to be

related to the chemical and topochemical natures of lignin and/or the nature of the lignin-carbohydrate association.

Hardwood lignin contains both guaiacyl and syringyl residues but softwoods lignin contains guaiacyl residue only[111]. Musha and Goring[95] demonstrated by ultraviolet microscopy that the walls of fibres and ray cells contain mostly syringyl residue, and that the vessel walls and cell corner regions contain mostly guaiacyl residue. Recently, Kirk et al.[73] reported that *C. versicolor* firstly degraded syringyl-rich lignin and then guaiacyl-rich lignin in the attack of birch wood (*Betula alleghaniensis*), through the progressive action of enzymes from the lumen surfaces toward the middle lamella. However, such successive degradation only reflects the distribution of lignin residues in birch wood, and does not show a greater susceptibility of the syringyl residue and hardwoods to this fungus. Although softwood lignin contains guaiacyl residue only, microscopic distribution of the residue in wood tissue and the association with carbohydrates probably vary with species at some extent. The mechanism of lignin degradation has been known to be largely oxidative but it has not been completely understood yet[71,74]. Knowledge about the depletion of lignin by soft rot fungi is especially meager. To define more accurately the significance of lignin in different decay resistance of wood, a further knowledge of factors varying with wood and fungal species is needed: such as chemical and topochemical natures of lignin, the nature of the lignin-carbohydrate association, topochemical effect of delignification, and fungal enzyme systems involved in breakdown of wood components.

## 5-2 Summary

The pattern of lignin removal from partially delignified woods by a soft rot fungus, *Chaetomium globosum* Kunze, and a white rot fungus, *Coriolus versicolor* Quél., has been studied on two softwoods, *Pinus densiflora* Sieb. et Zucc. and *Cryptomeria japonica* D. Don, and one hardwood, *Fagus crenata* Blume, with reference to the different acceleration pattern of wood decay caused by the partial delignification. The rapid and shorter acceleration, which was observed for the cases of *P. densiflora* and *C. japonica* attacked by *C. globosum* and *C. versicolor*, respectively, was accompanied with the rapid rate of lignin removal and the higher ratio of lignin loss to weight loss at smaller extent of delignification. The slow and longer acceleration, for the cases of *P. densiflora* and *C. japonica* attacked by *C. versicolor* and *C. globosum*, respectively, was accompanied with the slow or poor overall removal of lignin. In the former case, the lignin was removed apparently at slower rate than non-lignin components, and removal or modification of lignin probably acts largely for facilitating fungal enzyme systems to gain access to the carbohydrates. In the latter case, the ratio of lignin loss to weight loss increased in proportion to the extent of delignification, and reached the maximum level at the moderate extent of delignification. In the case of *F. crenata* which is highly susceptible to both fungi, the rate of lignin removal and the ratio of lignin loss to weight loss were always slower and smaller in *C. globosum* than in *C. versicolor*. On the basis of the results obtained, significance of lignin in different decay

resistance of woods against *C. globosum* has been discussed, comparing with the case of *C. versicolor*.

# CONCLUSION

Characteristics of wood decay by a soft rot fungus, *Chaetomium globosum* Kunze have been studied, with reference to the changes in physical- and chemical properties of wood, carbohydrate utilization by this fungus, decay resistance of various softwoods and hardwoods, and some factors concerning about different decay resistance among these woods.

Prior to these studies, investigations were made on the effects of variations in amount of carbon- and nitrogen sources, kind of carbon sources and size of test blocks, for seeking the cultural conditions which allow the higher wood-decaying capacity of this fungus. Results obtained suggest that wood-decaying capacity of this fungus is not exhibited in a nutritional condition which is favorable to brown rot- and white rot fungi.

It can be seen from the infrared spectral analysis of decayed wood that *C. globosum* is more active against hardwood lignin than is a brown rot fungus used, although preferential depletion of carbohydrates with little lignin attack has been emphasized as the similarity of soft rot to brown rot. Rapid decrease of the absorption at 1730 $cm^{-1}$ in *Fagus crenata*, assigned to the C=O stretching vibration of carboxyl and acetyl groups in glucuronoxylan, and the rapid utilization of xylose and hardwood xylan suggest a possible relation to the greater susceptibility of hardwoods to soft rot fungi. The ability of *C. globosum* to reduce the strengths of wood was rated in the middle of brown rot and white rot. This seems to reflect these degradation patterns of wood constituents

and the mode of attack on lignified cell wall which is generally characterized by cavity formation in secondary wall.

Decay resistance of various wood species (138 spp. of temperate hardwoods, 64 spp. of tropical hardwoods and 45 spp. of softwoods) against *C. globosum* was estimated with reference to methanol extractives in wood. With respect to temperate hardwoods, variances of decay resistance among species and families were not so much different from those for Basidiomycotina reported in the literature. In contrast with the case of temperate hardwoods, it was generally found that dense and/or extractive-rich species became more susceptible to decay after treatment with hot methanol. This was shown more notably in the case of white rot fungus, *Coriolus versicolor* Quél. Very low ability of *C. globosum* to degrade softwoods was evidenced for all wood species used. The greater part of species retained high resistance even after treatment with hot methanol. Also *C. versicolor* could not cause severe weight loss of softwoods. However, extractive-rich species were more resistant against this white rot fungus, and the greater part of these species became less resistant after extraction with hot methanol. It can be concluded from these results that the role of extractives in softwoods with higher resistance against soft rot fungi is generally insignificant.

Effects of some biological- and chemical treatments of softwoods on the wood-decaying capacity of *C. globosum* were investigated. Preexposure of softwoods to 9 Basidiomycotina and 1 Deuteromycotina did not facilitate attack by *C. globosum*. Attacking capacity of this fungus on softwoods was not enhanced by treatments with both mild alkali and acid.

However, treatment of softwoods with acidified sodium chlorite was very effective for acceleration of attacking capacity of this fungus. Such acceleration was found even in the case of lignolytic white rot fungi but not in the case of brown rot fungi. From the patterns of decay acceleration and lignin removal by soft rot- and white rot fungi, it can be concluded that lignin in softwoods is more or less hindrance in both types of wood rot fungi. With respect to a soft rot fungus, *C. globosum*, the hindrance by lignin may be rather qualitative than quantitative. However, for a white rot fungus, *C. versicolor*, the hindrance may act either qualitatively or quantitatively varying with wood species exposed. Quantitative hindrance is considered simply in terms of a physical blocking of the cellulolytic enzymes by lignin. Qualitative one seems to be related to the chemical and topochemical natures of lignin and/or the nature of the lignin-carbohydrate association, although knowledge of these natures is not fully available at present. To define more accurately the significance of lignin in different decay resistance of wood, a further knowledge of factors, including these natures, topochemical effect of delignification and fungal enzyme systems, is needed.

Besides in the way they attack wood, soft rot fungi have a number of distinctive physiological and ecological characteristics. Exemplified by *C. globosum*, they differ from other wood-decaying fungi in the way they modify the wood chemically and physically, resembling alternatively white rot fungi and brown rot ones yet behaving peculiarly. As has been mentioned, soft rot species such as *C. globosum* can attack hardwoods more easily than softwoods. However, partial delignification and split-

ting of the lignin-carbohydrate association, which is, by way of example, observed in wood cooling towers wetted by chlorine-containing water, will decrease the resistance of softwoods to soft rot. Similar effects are produced on wood surfaces by the physical and chemical factors of climate, and probably promote the initial invasion of soft rot fungi.

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